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SIR JOHN RUSSELL

FIRMAN E. BEAR

(WITH ONE PLATE)

Recently retired from the Directorship of the world-famous Rothamsted Experimental Station at Harpenden, Hertfordshire, some 25 miles out of London, Sir JOHN RUSSELL is now living at Woodstock, near Oxford, England. For 31 years he served with distinction as the administrative head of this renowned research institution, the Mecca of agriculturally-minded scientists from all quarters of the globe. Under his direction the Rothamsted laboratories, established a century ago by JOHN BENNETT LAWES, widely extended the range of their researches and contributed greatly to our knowledge of plants and animals, and of the soil upon which they depend.

EDWARD JOHN RUSSELL was born at Frampton, Gloucestershire, on October 31, 1872, the eldest son of the Reverend EDWARD T. RUSSELL. He was educated at University College of Wales, Aberystwyth, and at Victoria University, Manchester, and was later granted the D.Sc. degree from the University of London. He was elected a Fellow in the Royal Society in 1917 and achieved knighthood in 1922. Honorary degrees have been conferred upon him by the universities of Wales, Manchester, Toronto, Maryland, South Africa, Rutgers, Oxford, and Berlin. He was elected president of the International Society of Soil Science in 1930, completing his incumbency in that office at the end of the highly successful Soil Congress held at Oxford in 1935.

Following his university graduation, Dr. RUSSELL became successively lecturer in chemistry at Victoria University, 1898–1901; head of the chemistry department at the Agricultural College, Wye, 1901–1907; Goldsmith Company's soil chemist at Rothamsted, 1907–1912; and finally director of the Station, 1912–1943. In 1903 he married ELNOR OLDHAM of Manchester by whom he had six children, of whom four are living. One son, WALTER, is now soil-physicist on the Rothamsted Station staff.

Under Dr. RUSSELL's direction, new impetus was put into the study of soil microbiology, which was taken up where the pioneering work of PUGH and WARINGTON had left off some years earlier. Four lines of investigation were envisioned, *viz.*: algae, fungi, protozoa, and bacteria, and the accom-

plishments of Rothamsted for the last quarter-century were largely associated with this development. Early in this period, studies on soil sterilization by heat and volatile antiseptics were undertaken as a result of which it was found that the net effect of such treatments was a marked increase in bacterial activity. Out of this discovery grew the "protozoan theory of soil infertility," which was destined to become the center of a world-wide controversy. It assumed that bacterial development in the soil was kept in check by predatory protozoa that could readily be destroyed by partial-sterilization procedures.

Such enthusiasm for this theory was developed at Rothamsted that practically all of the Station's energies for many years were put into very detailed studies of the effects of partial sterilization on the soil microflora and microfauna. Some idea of the intensity of the attack on this problem is gained from the fact that, for a whole year, daily counts were made of the bacteria, amoebae, ciliates, and flagellates in the soil of certain of the field plots. In due time, however, the several microbiologists who had been assembled for these studies began to uncover more promising fields of study. Among these may be mentioned the researches on the nature of the aerobic bacteria concerned in cellulose decomposition; those on the balance between the nodule bacteria of legumes and their hosts; and those on nitrogen-carbon ratios required for rapid decomposition of organic matter in the production of synthetic manures.

To be a successful Director of the Rothamsted Experimental Station, as Sir JOHN unquestionably was, called for a degree of versatility few men possess. Thus the Station was constantly besieged by visitors from all over the earth, some of whom stayed on to work in the laboratories. Twice during his incumbency, England was faced with famine, as food shipments were cut off by the enemy submarines of World Wars I and II. In the intervening periods, ever greater demands were being made on his time, as requests for assistance came from the far-flung parts of the vast British Empire. Thus, although he began as a chemist, and became better known as a microbiologist, Dr. RUSSELL eventually qualified as an agriculturist, as his energies came to be directed more and more toward the broader aspects of the problems of food production.

In the memorable tour of the members of the International Soil Science Society in 1927, following the Soil Congress in Washington, Dr. RUSSELL was the outstandingly popular scientist of the group. At every stopping point on the tour across the United States and back by way of Canada it was he who was called upon to address the gatherings assembled at the banquets arranged by the local entertainment committees. Thus he possessed the capacity for adjustment that permitted of his being a democratic personality in America, but with an attractive trace of the blue-blooded Englishman that came to the fore when the occasion demanded. Certainly no one of the foreign visitors in this group of scientists understood America like Sir JOHN, for he had made lecture tours over the continent and had come to have

tremendous influence on the thinking of our state experiment stations, which are, in effect, copies of Rothamsted.

As an agriculturist, Dr. RUSSELL thinks of the soil, the microbes that live in it, the plants that grow on it, and the animals that consume its produce more as a critical scientist who sees promise of interesting discoveries than as one who runs his hands lovingly through the freshly plowed earth. Anyone who has taken the time to study his "Soil Conditions and Plant Growth," a book that has gone through six editions, and is the crowning publication of his career, will be impressed by the breadth of his scientific vision rather than by the down-to-earth quality of his writing.

A tremendous amount of energy is wrapped up in the body of this wiry, keen-minded man. Probably no other soil chemist and microbiologist has ever traveled so far, seen so many types of farming, and contacted so many research workers in their own laboratories as has Sir JOHN. And wherever he has gone, this restless energy has impelled him to look about in search of new truths in the field of agriculture. Many an associate has walked more miles across the fields, along country roads, or through city parks than he had intended because Dr. RUSSELL felt happier with his feet on the soil and his thoughts on the flora it supports. It is even rumored that he walked up Pike's Peak rather than go by automobile, but there are those who say he walked down instead.

Sir JOHN RUSSELL is widely known as a speaker on agricultural subjects, and when he speaks, men listen because he brings to bear on the subject a more-traveled and broader understanding than most men possess. Within recent years he has been devoting a great deal of time toward the bringing about of more friendly relations between the scientific workers in the field of agriculture in Soviet Russia and those in Great Britain. It is to be hoped that he may still find it possible to come this way again to tell us what is on his mind. In lieu of that, possibly some of us may be privileged to knock at his door, and enjoy a renewal of longstanding friendship. We wish him every happiness as he matures to a ripe old age in the peaceful little English village of his choice, overlooking the lovely agricultural region roundabout.

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CONCURRENCY OF CARBOHYDRATE FORMATION AND CARBON DIOXIDE ABSORPTION DURING PHOTOSYNTHESIS IN SUNFLOWER LEAVES

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(WITH TWO FIGURES)

Introduction

It was shown in a previous paper (**15**) that the amount of carbon dioxide absorbed by sunflower leaves during photosynthesis could be accounted for, under certain experimental conditions, by the increase in various carbohydrates. In all of the experiments there reported, approximately equal quantities of carbon dioxide were absorbed. The experiments have now been extended to include a greater range in the amounts of carbon dioxide absorbed during photosynthesis in order to determine to what degree carbohydrate formation, both individual and total, keeps pace with carbon dioxide absorption.

Methods

The methods of culture and of handling the leaves, the apparatus for measuring the absorption of carbon dioxide, and the procedures for determining carbohydrates used in the present experiments, were essentially the same as those used in the previous experiments (**15**). The experiments here recorded were performed at 20° C.

The effects produced on the carbohydrates of the leaf by varying the experimental conditions with respect to treatment with carbon dioxide and to illumination were determined. The conditions were varied as follows:

(a). No addition of carbon dioxide and no illumination;

(b). Addition of carbon dioxide and no illumination;

(c). Addition of carbon dioxide and illumination.

Under experimental conditions (a), the leaf half, which was placed in the photosynthesis apparatus, was maintained without illumination for about an hour in the carbon dioxide concentration naturally occurring in the apparatus. The carbon dioxide reached a concentration of about 0.2 per cent., owing to the respiration of the leaf and to the bicarbonate solution contained in the pH cell.

In experimental conditions (b), the leaf half was maintained for 45 minutes under the conditions just described for (a) and then subjected to a carbon dioxide concentration of about 3.7 per cent. for a period of 15 minutes. (The increased carbon dioxide concentration was produced by pipetting into the apparatus approximately 14.6 ml. (0°, 760 mm.) of carbon dioxide.) The changes in carbohydrates, as compared with control samples, brought about by these treatments are recorded in table I.

In experiments under (c), the leaf half, after standing in the photosynthesis apparatus for about 45 minutes and being treated with approximately

TABLE I

COMPARISON OF CARBOHYDRATE RECOVERY IN SUNFLOWER LEAF SAMPLES WITH AND WITHOUT TREATMENT WITH CARBON DIOXIDE IN THE DARK

Series number	1	2
Number of experiments	4	5
	Without CO_2 mg.*	With CO_2 mg.*
Monosaccharides	0.105 ± 0.061	0.081 ± 0.118
Sucrose	− 0.015 ± 0.048	0.076 ± 0.101
Sugar not identified	0.040 ± 0.079	− 0.311 ± 0.240
Polysaccharide not identified	− 0.046 ± 0.090	− 0.047 ± 0.090
Starch	0.005 ± 0.110	0.339 ± 0.152
Total soluble carbohydrate	0.089 ± 0.083	0.139 ± 0.177
Residue	0.115 ± 0.122	0.933 ± 0.301
Total recovery	0.204 ± 0.127	1.072 ± 0.283

* The change effected in the various fractions by the treatments indicated is expressed in terms of milligrams of carbon.

14.6 ml. of carbon dioxide for 15 minutes, was illuminated. In the experiments previously published (**15**), only this amount of carbon dioxide was absorbed during photosynthesis; in the present series of experiments, from one-half to three times this quantity was absorbed during the photosynthesis. In order that the initial concentrations of carbon dioxide would be approximately equal in all of the experiments, a fixed quantity of carbon dioxide (14.6 ml.) was always introduced initially into the apparatus. Whenever one-half of this quantity was to be absorbed, the full quantity of 14.6 ml.

TABLE II

PERCENTAGES OF CARBON ABSORBED DURING PHOTOSYNTHESIS RECOVERED IN DIFFERENT CARBOHYDRATE FRACTIONS. TEMPERATURE: 20.0° C.

Series number	1	2*	3	4
Number of experiments	5	7	4	5
Average time of illumination, min.	27 ± 1	58 ± 4	101 ± 8	146 ± 5
Average weight of carbon absorbed, mg.	4.115 ± 0.020	7.772 ± 0.038	15.412 ± 0.100	23.142 ± 0.190
	Percentage recovery of carbon absorbed			
	%	%	%	%
Monosaccharide	4.4 ± 4.1	10.0 ± 1.3	16.6 ± 1.3	22.3 ± 0.9
Sucrose	55.3 ± 3.5	51.8 ± 2.1	45.9 ± 4.3	39.9 ± 1.4
Sugar not identified	.....	3.1 ± 0.8	3.2 ± 0.4	1.1 ± 1.2
Polysaccharide not identified	− 7.7 ± 4.1	1.4 ± 1.0	3.3 ± 1.5	1.7 ± 1.9
Starch	36.5 ± 6.5	25.5 ± 2.2	27.8 ± 3.5	28.7 ± 1.7
Total soluble carbohydrate	86.6 ± 5.1†	91.9 ± 1.5	96.7 ± 2.7	93.7 ± 0.8
Residue	20.7 ± 5.4	6.5 ± 1.8	− 0.4 ± 1.8	− 1.5 ± 1.6
Total recovery	107.3 ± 4.6†	98.4 ± 3.1	96.4 ± 1.5	92.2 ± 1.3

* Data already presented in the previous paper (**15**).

† Values exclusive of the fraction "sugar not identified" which was not determined in all of the experiments.

was pipetted into the apparatus and illumination of the leaf was continued until the required amount of carbon dioxide had been absorbed. Whenever more than this quantity was to be absorbed, the additions beyond this amount were made in units of 14.6 ml., and each unit was absorbed before the succeeding one was added. The results of these experiments correlating carbohydrate formation with the uptake of carbon dioxide are given in table II, and figures 1 and 2.

The methods of carbohydrate analysis used in the previous investigation (**15**) were modified for use in a part of the experiments reported in this paper so that the hydrolysis of sucrose was effected by treatment with 1 N hydrochloric acid for 10 minutes rather than with 1 per cent. hydrochloric acid for 20 minutes. The hydrolyses were carried out at the temperature of a boiling water bath.

Results

The results obtained under experimental conditions (a), no addition of carbon dioxide and no illumination, are presented in table I, series 1. It is obvious that merely placing the leaf in the photosynthesis apparatus and allowing it to remain there for an hour had no significant effect on any of the categories of substances examined. Treatment of the leaf with carbon dioxide (approximately 3.7 per cent.) and without illumination, according to experimental conditions (b), did not significantly affect the soluble carbohydrates but did increase the "residue" very noticeably (table I, series 2). This increase was directly in line with the increases obtained at various stages of the carbon dioxide absorption (*cf.* fig. 2, curve 1).

The data obtained under experimental conditions (c), addition of carbon dioxide and illumination, are summarized graphically in figures 1 and 2. The amounts of different carbohydrates obtained (ordinates) and of carbon dioxide absorbed (abscissae) are expressed in terms of their carbon content. In table II are given the percentages of the carbon absorbed as carbon dioxide which were recovered in the various carbohydrate fractions. The percentages were calculated from the carbon content of the substances involved.

Under the conditions of the experiments, it was impossible to effect the absorption of more than about 43 ml. of carbon dioxide. When the leaves had carried on photosynthesis long enough to absorb this amount, they had become so flaccid, because of lack of external water supply, that they ceased to absorb any more carbon dioxide.

The results in table II show that, on the average, the percentages of carbon recovered as "total soluble carbohydrate" pass through a maximum as the amount of carbon absorbed increases. The maximum quantity of carbon recovered in these experiments as total soluble carbohydrate amounted to 96.7 ± 2.7 per cent. of the amount absorbed during photosynthesis. This value tallies well with the high recovery obtained previously (**15**) at 10.0°, 98.7 ± 2.1 per cent.

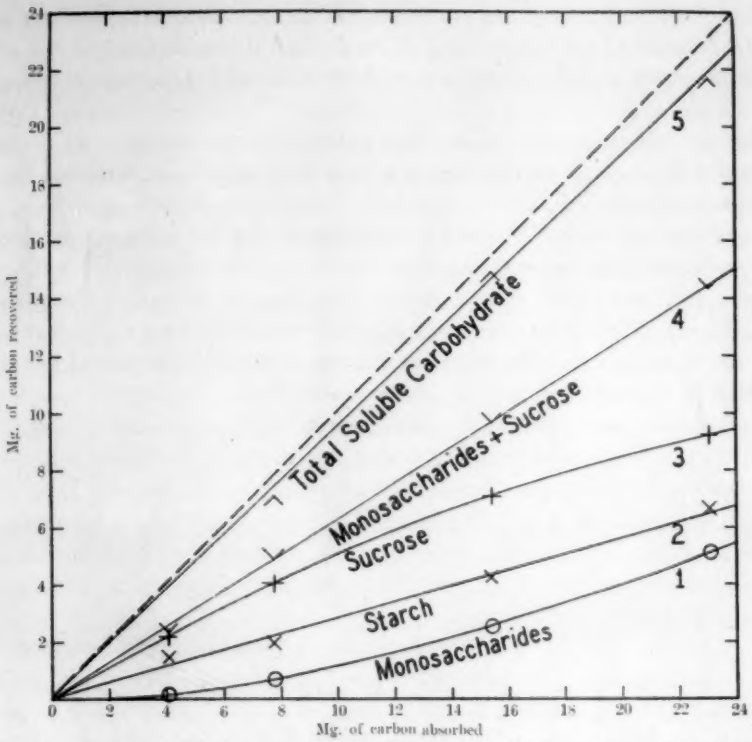

FIG. 1. Correlation of the amounts of carbon recovered in various carbohydrate fractions with the amount of carbon absorbed in photosynthesis by sunflower leaves. Temperature: 20.0° C.

When the experimental errors are taken into account, however, it is uncertain whether the percentages recovered as total soluble carbohydrate pass through a maximum, or remain constant over the range of carbon dioxide absorption investigated. How nearly a direct proportionality exists between the amount of carbon recovered as total soluble carbohydrate and the amount of carbon absorbed as carbon dioxide may be readily seen from the linearity of curve 5, figure 1.

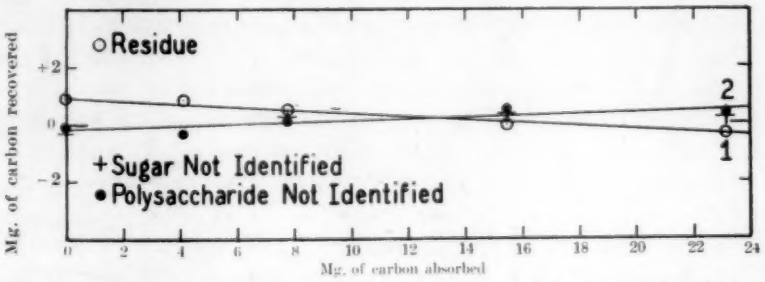

FIG. 2. Correlation of the amount of carbon recovered in various fractions of sunflower leaves with the amounts of carbon absorbed in photosynthesis by sunflower leaves. Temperature: 20.0° C.

The "total recovery," as percentage of carbon absorbed, declines as the carbon dioxide uptake increases. This decline is attributable to the steady decrease in the amount of carbon recovered in the "residue" fraction (*cf.* curve 1, fig. 2).

As the carbon dioxide absorption progresses, the percentages of carbon recovered as monosaccharides show a progressive increase, whereas the percentages recovered in the sucrose fraction show a progressive decrease. The changes are inversely related to each other so that the sums of the percentages recovered in these two classes are approximately constant over the range covered (*cf.* table II). The amounts of carbon recovered in the monosaccharide and sucrose fractions, and in the sums of the two, as related to the amounts of carbon dioxide absorbed (in terms of carbon content) are shown in figure 1, curves 1, 3, and 4. It is obvious from the curves that, as the carbon uptake becomes greater, the increase in the amounts of carbon recovered in the monosaccharide fraction becomes progressively greater but the increase in the amounts of carbon recovered in the sucrose fraction becomes progressively less. The sum of the two curves is a straight line, curve 4. This signifies that the amounts of carbon recovered in the monosaccharide plus sucrose fractions are directly proportional to the amounts of carbon dioxide absorbed.

It is noteworthy that the monosaccharides formed in photosynthesis consist of nearly equal quantities of ketoses and aldoses. In the two series of experiments representing the greatest amount of photosynthesis, ketoses comprised about 44 per cent. of the monosaccharides formed. Before photosynthesis, ketoses constituted about 8 per cent. of the monosaccharide fraction.

The amounts of carbon recovered as starch were also directly proportional to the amounts of carbon absorbed as carbon dioxide, as the straight-line nature of curve 2, figure 1, demonstrates. The approximate constancy of the percentages of carbon absorbed which were recovered in the starch fraction also attests to this direct proportionality.

The percentages of the carbon absorbed which were recovered as "sugar not identified" were approximately constant. With the exception of one value, the percentages of the absorbed carbon recovered in the fraction "polysaccharide not identified" were nearly constant also. The variability in the amounts of carbohydrates found in these two classes of carbohydrates were large as compared to the amounts formed. For this reason the course of synthesis of these carbohydrates is not well defined but the small increases observed are approximately proportional to the amounts of carbon absorbed as reference to figure 2, curve 2 shows. (The experimental points for these fractions are so close together that only one curve has been drawn for both sets of data.)

The behavior of the "residue" fraction is remarkable. As the amount of carbon dioxide absorbed in photosynthesis increases, the amount of insoluble residue progressively decreases until there is an actual loss of weight in this insoluble portion of the leaf (*cf.* fig. 2, curve 1). The nature and fate of this "residue" material are unknown.

Discussion

The high recovery of carbon found in these experiments, 96.7 ± 2.7 per cent., corroborates the recovery value, 98.7 ± 2.1 per cent., obtained in the previous experiments (**15**). These facts support the assumption that under certain circumstances nearly all, or possibly all, of the increase in organic matter produced through photosynthesis is carbohydrate. The possible deviation from complete recovery as carbohydrate and the uncertainties found in the recovery values would allow for the synthesis of small amounts of other substances. To demonstrate increases in such substances will require sensitive and characteristic tests.

The data obtained on the amounts of total soluble carbohydrates formed by photosynthesis from different amounts of carbon dioxide are not sufficiently precise to distinguish between the different paths that the carbohydrates in this category may follow. The data indicate that the percentages probably pass through a maximum as photosynthesis progresses; but the uncertainties would permit that they remain constant over the range covered. These two paths are the extremes and any trend between them is possible. To know the true path would aid in deciding what course carbohydrate synthesis follows. Whichever path is followed, it is obvious that in the early stages of photosynthesis only small amounts of material can possibly be diverted from carbohydrate synthesis.

Curves 2 and 4, figure 1, depict the amounts of starch and of sucrose plus monosaccharides formed by photosynthesis from different amounts of carbon dioxide. The linearity of these curves signifies that these products stand in constant proportion to each other. It can be shown that if several side-by-side chemical reactions of the same order are proceeding simultaneously from a common reactant, or reactants, then the ratio between the amounts of the different products formed remain in constant proportion to each other over the whole course of the reaction. Therefore, one interpretation of the experimental results is that the products, just named, arise concurrently from a common precursor through reactions of the same order.

An examination of curves 1 and 3, figure 1, shows that the initial rise in the sucrose curve is relatively rapid but that the initial rise in the monosaccharides curve is relatively slow. The fact that these curves are so related to each other that the sum of the two is a straight line suggests that the sucrose is formed prior to the monosaccharides. This suggestion is supported by the fact that the increase produced in the monosaccharide fraction during photosynthesis consists of both aldoses and ketoses in almost equal proportion, whereas the monosaccharides fraction before photosynthesis consists largely of aldoses. These observations may be interpreted as follows: starch and sucrose arise concurrently from a common precursor in side-by-side reactions, and the sucrose is subsequently inverted to aldose and ketose.

The increases in the fractions "sugars not identified" and "polysac-

charide not identified'' are, within the experimental error, directly proportional to the amounts of carbon dioxide absorbed (*cf.* fig. 2, curve 2).

The direct proportionality between the quantities of carbon dioxide absorbed and the amounts of various carbohydrates produced during photosynthesis strongly suggests that several of the carbohydrates are synthesized concurrently—perhaps from a common intermediate precursor—and do not arise *seriatim* as has often been proposed. Although it is possible to account in other ways for the observations presented here, the side-by-side course of carbohydrate synthesis offers a simple and direct explanation for the facts obtained in this investigation.

A rigorous treatment of the mechanism of the formation and transformation of the carbohydrates during photosynthesis would require a consideration of the various carbohydrates present in the leaf at the beginning of the photosynthesis period. No correlation between the increases of the individual carbohydrates brought about by photosynthesis and the amounts of the different carbohydrates present in the leaf at the beginning of the photosynthesis period has been found. The reason for the lack of correlation may be that the increase in any one carbohydrate is not dependent on the total amount of any carbohydrate already present in the leaf, but is dependent on only a portion of the carbohydrate contained within some specialized unit or present in some reactive form.

The results obtained from these experiments indicate that the starch and sucrose are formed concurrently in side-by-side reactions. Since the amount produced of each of these carbohydrates is a linear function of the amount of carbon dioxide absorbed during photosynthesis (taking into account the amount of sucrose transformed into monosaccharides) and since the curves representing the courses of both pass through the origin, it may be reasonably assumed that these carbohydrates come from a common intermediate precursor. What this precursor is, cannot be said at the present time. The experiments of HANES (**7**) on the synthesis of starch from glucose-1-phosphate, and the experiments of DOUDOROFF, KAPLAN, and HASSID (**6**) on the synthesis of sucrose from the same compound, suggest that this hexose-phosphate may be the common precursor[1]—at least these experiments demonstrate that one compound can act as a precursor for the synthesis of both starch and sucrose.

There has been a controversy of long standing as to whether starch, sucrose, or glucose is the first carbohydrate formed in photosynthesis (*cf.*

[1] The proposal that phosphate esters are intermediate compounds in the mechanism of carbohydrate formation in photosynthesis has been made by several investigators. BARRENSCHEEN and his co-workers have found that a reducing hexose-monophosphate, of the fructose series, increases regularly in Elodea during photosynthesis (**2**, **3**). BURKHARD and NEUBERG (**5**) have isolated from beet leaves a mixture of glucose- and fructose-monophosphate containing the two in equal amounts. HANES (**8**) has reported the presence of phosphorylases in leaves. And RUBEN (**11**) has postulated phosphorylation as an intermediate process in the absorption and reduction of carbon dioxide in the photosynthetic process.

STILES (**19**), SPOEHR (**17**), BARTON-WRIGHT (**4**), and HARTT (**9**). If it is confirmed by further work that starch, sucrose, and perhaps some of the other carbohydrates are formed concurrently from a common precursor, and that the glucose and fructose produced during photosynthesis arise from the inversion of sucrose, then some of the controversial questions of the *seriatim* sequence of carbohydrate formation in the photosynthetic process will cease to have any meaning. For example, if starch and sucrose arise simultaneously, it is futile to discuss which of these is the primary carbohydrate of photosynthesis. It may be conjectured that the extent to which the various carbohydrates are produced depends upon the availability of specific enzymes for transforming a common precursor along different paths.

Because of the behavior of the "residue" fraction with respect to treatment with carbon dioxide and to illumination, it might be assumed that this material is involved in the uptake of carbon dioxide in the dark (**10, 12, 14, 16**) and in photosynthesis. Indeed, this may be the case, but such an assumption is not yet justified on the basis of the experimental results. The increase in the residue fraction, brought about by treatment with carbon dioxide, should not be confused with the substances responsible for the absorption of carbon dioxide which have been distinguished previously by the use of radioactive carbon dioxide (**12, 13, 16**). These substances are water-soluble, whereas the increase in the residue fraction is insoluble in water.

Wilting of sunflower leaves is known to affect the transformations of carbohydrates therein (**1, 18**). In the present experiments, the water deficit occurring in the leaves during photosynthesis may have influenced the proportions of the various carbohydrates formed. The extent of this influence was not determinable from the results already published because of the difference in conditions under which the results had been obtained. An effort will be made to evaluate the effect of water deficit on the proportions of the various carbohydrates formed during photosynthesis.

Conclusion and summary

Sunflower leaves, during photosynthesis, accumulate an amount of carbohydrate equivalent to the amount of carbon dioxide absorbed. If other classes of substances are formed, they must accumulate in only very small quantities. Starch and sucrose plus monosaccharides increase in direct proportion to the amount of carbon dioxide absorbed during photosynthesis. The relationship between the amount of carbon recovered as starch and as sucrose plus monosaccharides indicates that starch and sucrose are formed concurrently in side-by-side reactions, and that the sucrose is subsequently inverted to monosaccharides. The possibility exists that sucrose and starch arise from a common precursor. Other carbohydrate fractions, which are formed in only minor amounts, apparently increase also in proportion to the amount of carbon dioxide absorbed. One fraction, the residue fraction, is increased by treatment of the leaf in the dark with carbon dioxide. This increase becomes progressively less as photosynthesis progresses until, in pro-

longed photosynthesis, an actual decrease in the residue is produced. If the course of the synthesis of the various carbohydrates from a common precursor, as presented here, is confirmed, a new aspect of the sequence of carbohydrate formation will have been gained.

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SOME EFFECTS OF BORON SUPPLY ON THE CHEMICAL COMPOSITION OF TOMATO LEAFLETS

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(WITH THREE FIGURES)

Introduction

Recent reports in the literature dealing with the effects of boron supply on plant growth have considered associated effects on the mineral composition of plants. Reports that boron supply may influence the accumulation or utilization of other elements have led certain investigators to postulate a regulatory rôle for boron in this respect (**10, 39, 52**). There is, however, a noteworthy lack of agreement among investigators as to the specific effect of boron on the accumulation of any given element. Some of this lack of agreement may be due to the use of a wide variety of crops by different workers and to the use of sand or solution cultures in some studies and various soils in others. There still remains, however, considerable disagreement in this respect among investigators who have worked with plants grown in soils, or among those who have used sand or solution culture techniques.

It has been reported that as the supply of boron to plants was increased either by fertilization of soils or by additions to nutrient cultures: the calcium content of plants or of various tissues was increased (**27, 28**), decreased (**14, 22, 27, 29**) or remained constant (**9, 26, 33**); the potassium content was increased, (**14, 21**) or decreased (**20**); the magnesium content was increased (**9, 41**), decreased (**20**), or remained constant (**9, 28**); the phosphorus content was decreased (**10, 11, 21, 53**), or unchanged (**33**); and the iron content was increased (**5**), decreased (**9, 29**), or remained constant (**28**).

Several investigators have pointed out that boron may be concerned with nitrogen and protein metabolism of plants (**32, 45, 46, 54**). Other results indicate that the protein or total nitrogen content of plant tissues increased (**8, 21, 28, 31, 36, 42**), decreased (**4, 11, 29, 44**), or was unchanged (**6, 34, 43, 50**) when boron supply to the plant was increased.

Several miscellaneous effects of boron supply have been noted which are pertinent to this discussion. An increased boron supply has been found to alleviate the deleterious effect of high potassium concentrations (**17**). An increased potassium supply, however, has alleviated (**40**) or accentuated (**38**) boron deficiency. It has also resulted in an increased (**38**) or decreased (**7**) boron content in the plants. Other workers (**23, 25, 47, 55**) have noted the existence of a boron-calcium relationship in the nutrition of plants, and the suggestion has been made that boron is involved in the regulation of a calcium-potassium interaction (**40**). Also, the effects of increased iron supply in alleviating boron toxicity (**14**) and of increased boron supply in alleviating iron toxicity (**24**) have been pointed out. Studies of the ash con-

tent of plants have shown that an increased boron supply is associated with a decreased ash content (**4, 11, 15**), an increased ash content (**25**), or unchanged ash content (**27**). Increased (**27**) and decreased (**12**) percentage dry matter has been reported with an increased boron supply to the plant.

The literature, therefore, shows striking disagreement concerning the effects of boron supply on the mineral content of plants. Many of the effects noted are of a sufficiently great order of magnitude to be of importance both from the standpoint of the nutritive value of food crops and from the standpoint of plant nutrition. The present study was undertaken in an attempt to determine, under carefully controlled conditions, the effects of wide variations in boron supply on the chemcial composition of tomato leaflets. The treatments were designed to include rates of boron supply which would produce plants exhibiting symptoms of a severe boron deficiency, plants with symptoms of severe boron toxicity, and plants of apparently normal boron nutrition. In this investigation, the contents of fourteen of the essential nutrient elements have been used as criteria of chemical composition.

Materials and methods

Two experiments were conducted at Ithaca, New York, during the summer of 1943. In experiment 1, seeds of an inbred strain of Bonny Best tomatoes were planted in the greenhouse May 1. The seeds were germinated in small crocks filled with pure quartz sand and supplied with a nutrient solution of the following composition (p.p.m.): Ca, 285; K, 223; Mg, 69; N, 168; S, 144; P, 70; Fe, 5.0; Mo, 0.05; Mn, 0.5; Zn, 0.05; Cu, 0.02. This solution will hereafter be referred to as basal nutrient. Eighteen seedlings were transplanted into two-gallon glazed crocks with one seedling per crock on May 20, and all plants were placed outdoors. Nine plants were supplied with the basal nutrient throughout their life span; the remaining nine plants were supplied with the same nutrient solution containing in addition 0.5 p.p.m. of B. Three plants of each treatment were randomized as to position in each of three rows. A total of nine plants was used in each treatment.

The crocks and plants were supported by stakes. All axillary growth of each plant was pruned off twice weekly. During the early growth stages, nutrient solution was supplied to the plants three times each week. As the summer progressed, nutrients were supplied every other day, and finally—when fruits were ripening—they were applied four times per week. The solutions were applied in 500-ml. quantities which were sufficient to insure an appreciable amount of drip, and each pot was watered with distilled water between nutrient applications. The first four fruits to ripen on each plant were picked on the morning of the day that complete color change had occurred and analyzed for vitamin content. All plants were harvested August 21 when they were 112 days old.

At the time of harvest the height of each vine and the number and total fresh weight of immature fruit on each plant were recorded. The leaflets of each plant were subsequently separated from the petioles and weighed.

The remaining parts of the vine were then harvested and the root systems washed free of sand. The leaflet, vine, and root materials of each plant were dried at 70° C. for 72 hours and dry weights recorded. The nine individual leaflet samples of each treatment were preserved for chemical analyses, the results of which are reported here.

In experiment 2, seeds of the same inbred strain of Bonny Best tomato were germinated in the greenhouse May 5, using the same equipment and base nutrient solution as in experiment 1. On May 25, fifty seedlings were transplanted into large crocks and placed outdoors. Five treatments with ten plants each were used which differed only in the amount of boron supplied to the plants in the basal nutrient solution. The boron concentrations (p.p.m.) were as follows: 0.5, 15.5, 30.5, 45.5, and 60.5. The respective treatments were started June 5 when the plants were 31 days old.

A randomized block design was used (**13**) with five replications (blocks). Each treatment consisted of a two-plant row in each block, and a total of ten plants was used in each treatment. The treatments were randomized as to position within a block by the use of TIPPETT's randomization tables (**49**).

The methods of supporting the plants, pruning, supplying nutrients, and harvesting ripe fruit for vitamin analyses were the same as for experiment 1. The plants were continued on their respective treatments until August 26 when they were 113 days old. Finally at harvest, the same procedure as in experiment 1 was used. The leaflets of the two plants in each block which received the same treatment were composited, and thus five leaflet samples or replications were available in each treatment for the purpose of this investigation.

Each leaflet sample was ground in a mortar and pestle to prevent mineral contamination (**16**), and duplicate analyses for K, Ca, Mg, S, Na, P, Mo, Cu, Mn, Zn, Fe, and Co were made by the method of PARKS *et al.* (**35**). Boron was determined by the method of NAFTEL (**30**); and organic-N (**1**), NO_3-N (**51**), and NH_3-N (**1**) were determined by the indicated methods. The mean of the duplicate analyses for any given element was used for each leaflet sample, the treatment means computed, and all differences between treatment means tested for statistical significance by the *t* test (**48**).

Results

Measurements of the growth and fruitfulness of the plants in both experiments are given in table I. In experiment 1, plants supplied with nutrient solution lacking boron were significantly smaller with less extensive root systems than those plants supplied with 0.5 p.p.m. boron in the nutrient medium. For convenience the former plants will be designated as boron-deficient and the latter as normal or control plants. Fruitfulness was also significantly less in boron-deficient plants. Mean differences in all characters except percentage dry matter of vines are highly significant statistically. In addition, severe deficiency symptoms similar to those obtained in other investigations were observed on vegetative parts of the plant (**18, 19**) and on fruits (**19, 37**) in the boron-deficient treatments.

TABLE I

MEASUREMENTS OF GROWTH AND FRUITFULNESS, GIVING TREATMENT MEANS TOGETHER WITH THEIR STANDARD ERRORS

TREATMENT (BORON SUPPLY)	GROWTH					FRUITFULNESS				
	HEIGHT OF VINE	FRESH WEIGHT OF VINE	DRY WEIGHT OF VINE	PERCENTAGE DRY MATTER	DRY WEIGHT OF ROOTS	TOTAL FRESH WEIGHT RIPE FRUIT	NO. OF FRUIT RIPENED PER VINE	SIZE OF RIPE FRUIT	NO. OF IMMATURE FRUIT	TOTAL FRESH WEIGHT IMMATURE FRUIT
p.p.m.	*cm.*	*gm.*	*gm.*	%	*gm.*	*gm.*		*gm./fruit*		*gm.*
A. Experiment 1										
0.0	67.7 ± 5.74	200.5 ± 23.10	37.1 ± 4.60	18.2 ± 0.75	4.7 ± 0.49	331.4 ± 106.71	4.9 ± 1.21	65.9 ± 14.77	0.2 ± 0.22	4.3 ± 4.30
0.5	109.6 ± 4.39	356.0 ± 18.63	68.7 ± 3.18	19.4 ± 0.50	9.0 ± 0.70	831.7 ± 63.76	9.7 ± 0.81	88.5 ± 6.91	9.3 ± 1.38	499.1 ± 70.48
B. Experiment 2										
0.5	101.5 ± 4.28	271.3 ± 21.69	47.9 ± 1.46	17.7 ± 0.28	8.4 ± 0.35	774.2 ± 49.51	6.9 ± 0.82	119.7 ± 9.59	10.4 ± 1.44	523.9 ± 52.24
15.5	99.6 ± 5.21	176.6 ± 19.46	26.4 ± 1.75	15.1 ± 0.65	4.2 ± 0.23	621.8 ± 26.18	6.6 ± 0.45	97.3 ± 6.10	5.6 ± 1.19	143.1 ± 28.42
30.5	88.7 ± 5.11	105.3 ± 13.73	14.7 ± 1.74	14.2 ± 0.44	3.3 ± 0.47	456.3 ± 30.86	6.1 ± 0.53	76.7 ± 3.85	1.0 ± 0.56	6.8 ± 2.45
45.5	78.8 ± 6.10	66.3 ± 14.76	8.4 ± 1.34	13.7 ± 0.73	1.8 ± 0.20	354.7 ± 29.38	5.3 ± 0.52	69.7 ± 5.83	0.1 ± 0.10	2.4 ± 2.40
60.5	55.3 ± 3.76	29.7 ± 2.35	4.0 ± 0.28	13.7 ± 0.47	1.2 ± 0.08	230.5 ± 26.19	3.7 ± 0.37	63.6 ± 7.51	0.4 ± 0.22	5.3 ± 3.80

The data in table I show that significantly less growth and fruitfulness were associated with increased boron supply in experiment 2. Marked toxicity symptoms occurred on plants supplied with the four highest concentrations of boron, and the severity of the symptoms increased with increased boron supply. In this experiment the plants receiving 0.5 p.p.m. boron in the nutrient medium will be designated as control plants while those treatments using nutrients containing greater quantities of boron will be designated as toxic. The symptoms as well as the data concerned with growth and fruitfulness of the plants and results of vitamin analyses will be discussed in a subsequent paper.

The results of all chemical analyses are given in table II. Treatment means, together with their standard errors, are presented to aid in evaluating the statistical significance between treatments. In treatments where the amount of material available for analysis was limited, replicates were combined, and only the mean of duplicate analyses on a composite sample is available. It is not possible in these instances to use such values in a statistical test since no standard errors are presented. It is noteworthy that significant differences between treatments exist with respect to all elements except sodium.

The concentration of each element in leaflet material is presented graphically as a function of boron supply in figures 1, 2, and 3. In each graph the broken base line and the discontinuous nature of the curve indicates that results from two separate experiments differing in environmental variables other than nutrient supply were utilized. In noting the lack of coincidence of some analytical results of control plants in experiments 1 and 2, it should be recalled that differences in uncontrolled environmental factors also resulted in some differences in growth and fruitfulness (table I).

For convenience, boron supply is presented in different abscissal units for graphing results from experiments 1 and 2. In the curves the reliability of any point may be estimated from standard errors of means presented in table II. The unreliability of those points which represent results from composited samples is indicated by dotted portions of the curve.

Increasing boron supply resulted (fig. 1) in marked increases in the boron content of tomato leaflets. The standard errors and the number of degrees of freedom involved in each mean (table II) clearly show the differences in boron content from deficient to normal and from normal to toxic levels of boron supply are all highly significant. The boron contents of leaflets from plants grown in deficient and toxic treatments are in accord with other results (**2**) and with the observed deficiency and toxicity symptoms.

Protein content increased as the boron supply was increased (fig. 1). In experiment 1, the crude protein content of the plants receiving a normal (0.5 p.p.m.) supply of boron was significantly higher than that of the boron-deficient plants, and the plants receiving toxic (15.5 p.p.m. and higher) concentrations of boron (experiment 2) were significantly higher in organic

TABLE II

EFFECTS OF BORON SUPPLY ON THE CHEMICAL COMPOSITION OF TOMATO PLANTS

TREATMENT (BORON SUPPLY)	MILLIGRAMS PER GRAM DRY WEIGHT OF LEAFLETS								
	Ca	Mg	K	Na	P	S	Org.-N	NO_3-N	NH_4-N
p.p.m.	*mg./gm.*	*mg./gm.*	*mg./gm.*	*mg./gm.*	*mg./gm.*	*mg./gm.*	*mg./gm.*	*mg./gm.*	*mg./gm.*
A. Exp. 1									
0.0	39.4 ±* 1.78 (7)	3.7 ± 0.23 (7)	24.3 ± 0.99 (7)	1.9 ± 0.45 (7)	8.2 ± 0.24 (7)	15.0 ± 1.49 (7)	25.2 ± 0.54 (7)	0.31 (2)	0.2 (2)
0.5	55.3 ± 1.42 (9)	9.2 ± 0.56 (9)	18.3 ± 1.29 (9)	2.2 ± 0.16 (9)	7.2 ± 0.14 (9)	26.2 ± 1.36 (8)	29.0 ± 0.59 (9)	0.74 (2)	0.3 (2)
B. Exp. 2									
0.5	62.7 ± 1.23 (5)	6.9 ± 0.05 (5)	14.0 ± 0.71 (5)	2.0 ± 0.34 (5)	8.8 ± 0.17 (5)	25.1 ± 1.12 (5)	27.4 ± 0.25 (5)	0.14 (1)	0.4 (2)
15.5	48.7 ± 1.41 (5)	5.1 ± 0.21 (4)	21.3 ± 0.95 (4)	1.3 ± 0.24 (4)	11.6 ± 0.55 (5)	16.3 ± 1.05 (4)	38.9 ± 0.54 (5)	0.21 (2)	0.2 (2)
30.5	41.2 ± 1.32 (5)	3.9 (1)	17.0 (1)	0.2 (1)	14.2 ± 0.50 (4)	10.0 (1)	45.2 ± 0.42 (5)	0.28 (2)	0.2 (2)
45.5	38.4 ± 1.24 (3)	.....	.....	.....	13.7 ± 1.88 (3)	.....	49.0 ± 0.32 (3)	.....	0.2 (1)
60.5	39.6 (1)	.....	.....	.....	15.2 (1)	.....	.....	.....	.....

* The treatment means are reported together with their standard errrors. Duplicate analyses were made on material from each replication and the number of replications are indicated in parentheses.

TABLE II (*Continued*)

Treatment (boron supply)	Micrograms per gram dry weight of leaflets						
	Mo	Fe	Mn	Co	Zn	Cu	B
p.p.m.	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*	*μg./gm.*
A. Exp. 1							
0.0	7.0 ± 0.38 (7)	460 ± 31.7 (7)	146 ± 7.4 (7)	0.26 ± 0.008 (7)	31.6 ± 2.38 (7)	18.6 ± 0.86 (7)	7.6 ± 1.19 (9)
0.5	1.1 ± 0.10 (9)	542 ± 48.3 (9)	169 ± 16.4 (9)	0.31 ± 0.017 (8)	16.8 ± 1.19 (8)	12.3 ± 0.64 (8)	91.8 ± 3.30 (9)
B. Exp. 2							
0.5	1.2 ± 0.03 (5)	277 ± 7.4 (5)	123 ± 3.4 (5)	0.18 ± 0.014 (4)	21.8 ± 1.51 (5)	11.8 ± 0.55 (5)	147 ± 18.5 (5)
15.5	26.0 ± 0.73 (5)	377 ± 11.2 (5)	176 ± 5.5 (5)	0.25 ± 0.017 (4)	33.9 ± 0.87 (4)	21.0 ± 1.00 (4)	982 ± 87.7 (5)
30.5	4.9 ± 0.34 (5)	438 ± 19.7 (5)	184 ± 7.4 (5)	0.35 (1)	51.9 (1)	22.0 (1)	1351 ± 101.1 (5)
45.5	3.6 ± 0.25 (3)	447 ± 35.2 (3)	203 ± 15.2 (3)	.....	.....	.....	.....
60.5	3.1 (1)	439 (1)	252 (1)	.....	.....	.....	.....

nitrogen than the plants receiving a normal boron supply. The concentrations of ammonia and nitrate nitrogen present in these samples (table II) were very low and essentially constant for all treatments.

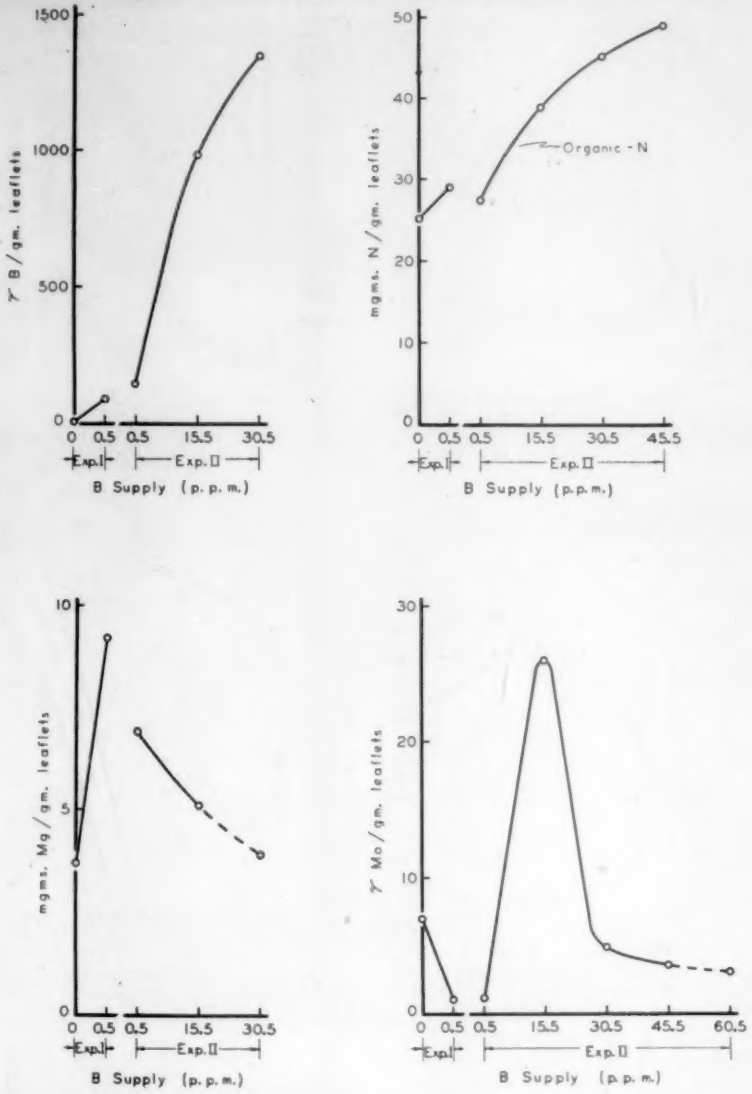

FIG. 1. The effects of boron supply on the boron, nitrogen, magnesium, and molybdenum concentrations in tomato leaflets.

The plants grown with a normal boron supply had more than twice the magnesium content of the boron-deficient plants on a percentage or concentration basis. On a per plant basis, the difference would be even more striking owing to the increased yield at the normal level. There is a cor-

responding, though less marked, decrease in magnesium content with higher levels of boron supply. Trends in composition similar to that for magnesium would be accentuated if computed as content per plant, since trends in yield are similar to those shown for concentration.

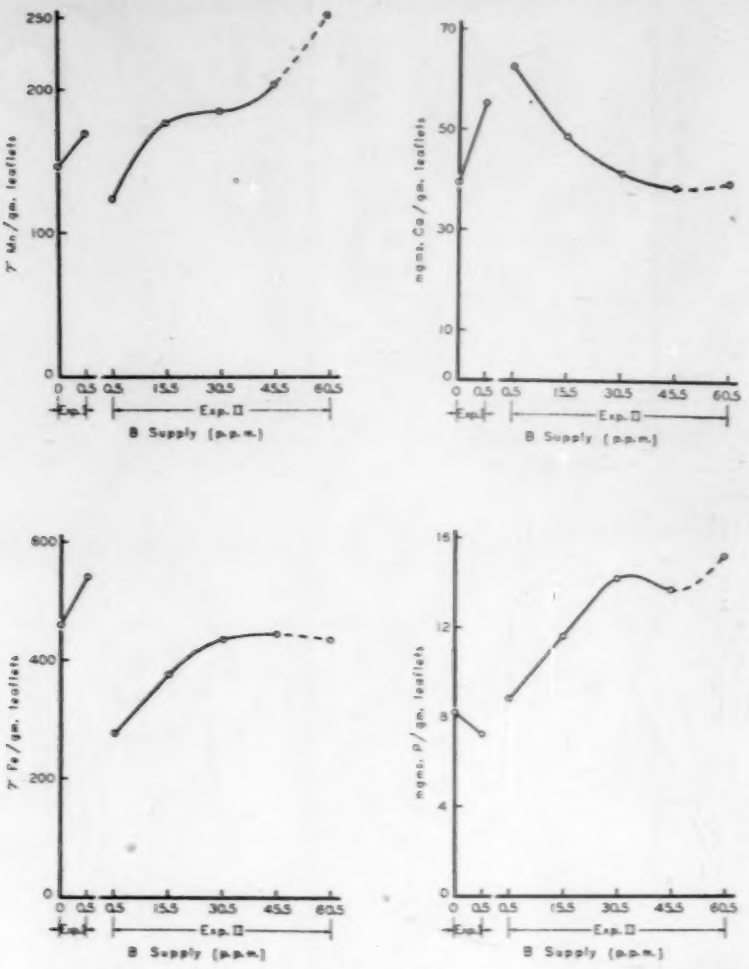

FIG. 2. The effects of boron supply on the manganese, calcium, iron, and phosphorus concentrations in tomato leaflets.

The concentration of molybdenum in these samples (fig. 1) was affected more strikingly by changes in boron supply than was that of any other element. Particularly noteworthy is the very high molybdenum content of plants grown in solutions containing 15.5 p.p.m. boron and the difference in molydbenum content between deficient and normal plants. A t test using the values and standard errors in table II shows these differences to be highly significant.

The responses shown with respect to the remaining nine elements might be grouped to show similarity in trends. Like boron and protein content (fig. 1) the concentration of manganese and iron (fig. 2), and cobalt (fig. 3) increase with increased boron supply in each experiment. A source of cobalt was not intentionally supplied in the nutrient medium. Analyses for cobalt in the samples were made since maladies have been associated with a lack

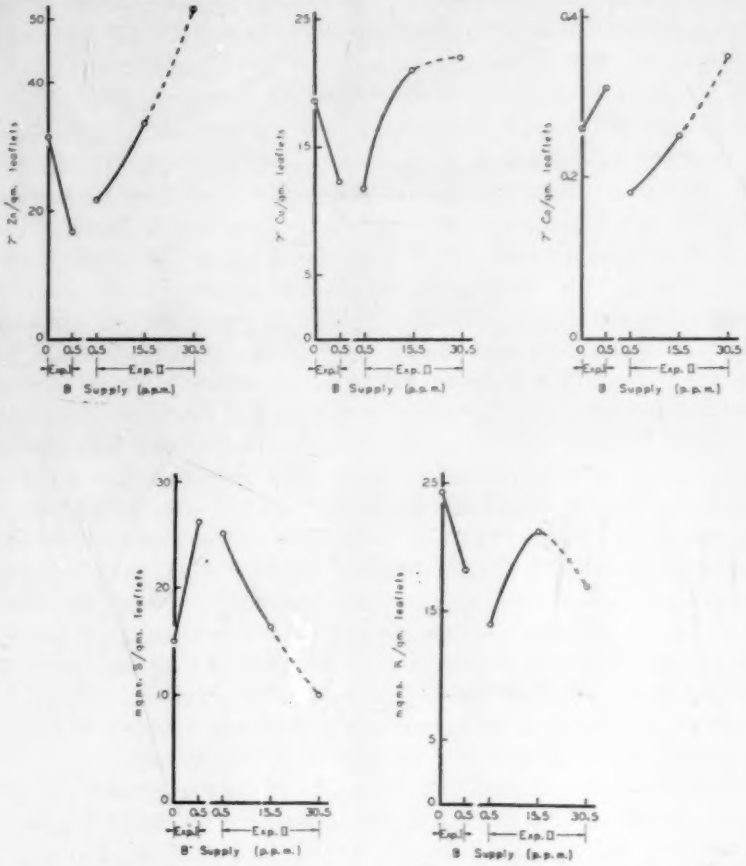

FIG. 3. The effects of boron supply on the cobalt, zinc, copper, sulfur, and potassium concentrations in tomato leaflets.

of cobalt in nutritional studies. The curve showing cobalt content of leaflets graphed as a function of boron supply is presented as a matter of theoretical interest and is based on the assumption of a constant cobalt supply as an impurity present in other salts or as an impurity contributed by the sand substrate. The validity of such an assumption is problematical. A similar assumption would be necessary for sodium. No statistically significant differences occurred, however, between treatments with respect to sodium content of the leaflets, and no graph is presented for this element.

Like magnesium (fig. 1), the maximum concentration of calcium (fig. 2) and sulfur (fig. 3) was found in control treatments of both experiments. Boron supplies of less than 0.5 p.p.m. or more than 0.5 p.p.m. were associated with a significantly lower concentration of these elements.

A minimum concentration of phosphorus (fig. 2), zinc, copper, and potassium (fig. 3) occurred in leaflets from control plants, and the trends shown are the reverse of those for the magnesium group. In boron deficient or toxic treatments the concentration of these elements was significantly greater.

Discussion

It is clear from the results reported here that differences in boron supply have resulted in differences in the boron content of plant tissue. In the same plant tissue differences in the concentration of most of the elements considered in this investigation were obtained. Since the nutrient composition of the medium was constant with the exception of boron in all treatments, it seems clear that a differential rate of absorption must have occurred, unless differences in boron supply merely resulted in a redistribution of mineral elements among plant organs. Existing evidence would indicate the former situation to be the more likely. In either case, however, altered mineral content as a result of varied boron supply would be of fundamental importance in plant nutrition and in evaluations of the nutritive quality of food crops. If differential absorption occurred, the cause is not known. It obviously cannot be directly attributed to differences in total growth of the plants or to relative extent of root systems since, if such were the case, uniform trends in content of the different elements would have to exist. The data presented here do not support this contention. At least, similar trends with respect to any given element would have to be associated with growth differences in different experiments. Such a situation does not exist in these and other data. For instance, in this experiment maximum calcium content was associated with maximum growth, and in another experiment (**3**) maximum growth was associated with minimum calcium content.

The trends reported here do not seem to be associated with any particular type of ion. For instance, opposite trends can be found among cations (Ca and Cu) and among anions (P and S). In addition, opposite trends are evident among divalent ions (Ca and Zn), and no correlation of trends with the valence of ions was observed.

Although tomato leaflets are not consumed directly by animals, they represent the leafy portion of a vegetable crop. In the case of forages and many vegetables, the chemical composition of this leafy material is important nutritionally for herbivora and man. Thus, the trends shown in tomato leaflets may serve as a basis to examine effects which may be of value in other plants. For instance, in this experiment protein content of leaflets varied approximately 100 per cent. A decreased yield of dry matter, however, was also obtained in treatments supplied with toxic quantities of boron, and these treatments were high in protein. If somewhat greater protein content

resulted from an increased boron supply with little or no suppression of yield in a forage crop, it would have profound significance nutritionally. That such a situation might result in increased protein per plant in the case of tomatoes can be shown by an interpolation of yield and protein curves between the first two treatments of experiment 2.

It seems possible that some of the discrepancies to be found in published data might be explained by trends reported here. For instance, reports of increased magnesium content associated with increased boron supply are supported by these data if the levels of boron supply were in a low range. Reports of decreased magnesium content associated with an increased boron supply are supported by these data if the level of boron supply was in a relatively high range. Similarly, MINARIK and SHIVE (**27**) have noted that maximum calcium content is associated with optimum boron supply.

Results of investigations concerned with ionic interaction have been reported from this laboratory (**3**). In these studies calcium ion supply was significantly and positively correlated with calcium content of tomato leaflets. In the same treatments potassium ion supply was significantly and negatively correlated with calcium content of leaflets. Therefore, the net effect (calcium content of leaflets) was associated at least with both calcium and potassium ion supply. Similarly, a magnesium-calcium interaction was associated with magnesium content of leaflets, and a magnesium-phosphorus interaction was associated with phosphorus content of leaflets. The fact that differences in boron supply have resulted in differences in plant composition with respect to nearly all the elements examined suggests that boron may be a component of one or more interactions or that complex interactions involving more than two elements may exist.

Summary

1. The effects of boron supply, ranging from deficient to toxic concentrations, on the chemical composition of tomato leaflets were examined. Results from two experiments using plants grown in sand culture are reported. The concentrations of K, Ca, Mg, S, Na, P, N, Mo, Cu, Mn, Zn, Fe, Co, and B were used as criteria of chemical composition.

2. As boron supply was increased, the concentration of this element in leaflet material was significantly increased.

3. There were also significant and large differences between treatments with respect to the concentration of most of the other elements examined, as boron supply was increased. The concentration of some elements was altered as much as several hundred per cent.

4. The results reported offer a possible explanation for confusion which exists in the literature. For instance, reports of trends involving increased magnesium, calcium, or potassium concentrations, or decreased magnesium, calcium, or potassium concentrations associated with increased boron supply could all be supported by these data if one assumes different initial levels of boron supply.

5. It was evident that boron supply had specific effects with respect to different elements, since the trends shown in plant composition for varying boron supply were completely dissimilar for different elements.

6. Differences between trends shown for various elements could not be correlated with the type of ion (cation or anion), the valence of ions, or total growth of the plants.

7. The possible importance of these effects with respect to plant nutrition and to the nutritive value of food crops is discussed.

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NUTRIENT INTERRELATIONS IN LIME-INDUCED CHLOROSIS

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Introduction

Chlorosis, or a low chlorophyll concentration in plants, may be brought about by many factors. Light or temperature conditions, hereditary factors, pathogens, and nutritional derangements are all recognized as causing such a condition.

Disturbances in iron nutrition are probably the most commonly recognized nutritional disorders causing chlorosis. There are various ways in which the iron nutrition of a plant may be affected so as to bring about a chlorotic condition. Four of the more commonly recognized types are: (1), true iron deficiency; (2), an upset in the phosphate/iron balance; (3), an upset in the manganese/iron balance; and (4), lime-induced chlorosis. The latter is probably the most widespread disorder of this type, and it assumes serious proportions in the more arid regions of this country where high concentrations of carbonates of calcium and magnesium are present in the soil. In some localities, lime-induced chlorosis is considered to be the primary limiting factor in the production of certain tree fruits. Present control practices are generally temporary and far from satisfactory. If a better understanding of the physiology of this disorder could be attained, more efficient methods of control might be developed.

The other iron disorders mentioned above are not known to be directly associated with lime-induced chlorosis. True iron deficiency occurs experimentally in water and sand cultures when the iron level of the nutrient solution is kept low. The iron content of such chlorotic plants, when expressed on an appropriate basis, is naturally lower than that of normal green plants. Since iron is so widely distributed in most soils and since the amounts of iron necessary for normal plant growth are relatively small, this simple type of chlorosis probably occurs infrequently under field conditions.

The phosphate/iron balance in relation to chlorosis has been discussed at length by Olsen (**19**). He has shown that plants grown in water cultures relatively high in phosphates, with iron supplied as the ferric ion, became chlorotic even though the total iron content of the leaves of the chlorotic plants was as much as, or more than, the iron content of green leaves. The chlorotic plants absorbed more phosphates, and Olsen concluded that iron was precipitated as phosphate in the leaf tissue, particularly along the vascular bundles. This chlorotic condition was overcome either by reducing the phosphate supply or by increasing the calcium. Olsen also showed that in water cultures the pH of the solution was effective in inducing chlorosis through its effect on the absorption of phosphates. Plants grown in solutions of pH 6 to 7 absorbed more phosphates and were chlorotic, while plants in solutions of either higher or lower pH were green. When iron was supplied in slightly ionized form (ferric citrate), the plants,

regardless of the pH, did not absorb sufficient phosphates to become chlorotic. CLARK (**5**) has reported somewhat similar effects. It is conceivable that this type of chlorosis may possibly occur in the field where heavy applications of phosphates are made, although so far as the writers are aware, no cases of chlorosis have been definitely correlated with this cause.

In Hawaii, where soils are high in manganese, a chlorotic condition of plants occurs that can be overcome by the addition of iron. RIPPEL (**25**) and MARTIN (**15**) have shown that a high manganese level in water cultures will induce chlorosis and that this condition could be overcome by increasing the iron level of the solution. Since the iron content of normal and chlorotic plants was found to be approximately the same, RIPPEL concluded that manganese affected the iron metabolism within the tissues of the plant. More recently, further studies of this manganese/iron balance in plants have been reported (**27, 28, 29**). These have shown that if the manganese concentration is too high, a typical iron chlorosis is produced which can be controlled either by increasing the iron level of the nutrient or by reducing the manganese level. The absolute amounts of these elements (over a fairly wide range) are immaterial as long as the proper balance exists between them. They concluded that "high concentrations of soluble manganese in the tissues are invariably associated with low concentrations of soluble iron and vice versa. This suggests the oxidation of ferrous to ferric ions by active manganese, in the inactivation and precipitation of iron in the form of ferric organic complexes."

Lime-induced chlorosis was thought at one time to have been due to a high calcium carbonate content of the soil raising the pH of the soil to such an extent that iron was made unavailable to the plant. When it was discovered that the iron content of normal and chlorotic plants was essentially the same (**1, 16, 17, 21, 32**), this hypothesis was no longer tenable. Since chlorotic leaves turned green when they were sprayed with iron compounds, the only possible conclusion seemed to be that iron in the chlorotic plant was present in an inactive form. OSERKOWSKY (**21**) first explored this avenue of approach. He found that during the early part of the growing season, the iron content of the 1 N HCl extract of pear leaves was in proportion to the chlorophyll content of the leaves. He concluded that a part of the iron in this extract was the "active" iron in chlorophyll formation. Since iron is not known to be part of the chlorophyll molecule, it is assumed that iron acts in a catalytic manner in chlorophyll formation, presumably as part of an enzyme. Thus the "active" iron of OSERKOWSKY might correspond to the iron in the enzyme that is responsible for chlorophyll formation.

It is the purpose of this paper to discuss some of the complex nutrient interrelations of lime-induced chlorosis and their effect on the iron metabolism of the pear and of certain other plants. While this work is only exploratory in nature, it is hoped that it may lead to a better understanding of the physiology of this disorder.

Methods

Analytical procedures

Surface contamination of leaf samples with dust is a major source of error in iron determinations and it was necessary to wash all samples. This was accomplished by swabbing each leaf with moist cotton followed by rinsing in distilled water. The samples were subsequently dried in a vacuum oven at 80° C.

One-gram samples were weighed out and ashed in a muffle furnace at about 450° C. After cooling, the ash was moistened with a small amount of water and then dissolved with 5 ml. of 6 N nitric acid. The mixture was brought to a boil to oxidize any ferrous iron to the ferric form. On cooling, the solution was filtered through #40 Whatman paper which had previously been washed with 6 N nitric acid and water. The filtrate was collected in a 50-ml. glass-stoppered volumetric flask and made to volume with water. When magnesium and phosphorus were to be determined on the same sample, it was necessary to add some alkaline material which did not contain magnesium, but which prevented the volatilization of phosphorus. A mixture of 4 grams of sodium nitrate and 1 gram of sodium hydroxide dissolved in 100 ml. of water was found to be satisfactory for this purpose. Five ml. of this solution was used per gram of material and the mixture was evaporated before ashing. If, after several hours' ashing, carbonaceous material was still present, the sample was cooled, moistened with a small amount of water, and 1 ml. of 6 N nitric acid added. After drying, the sample was re-ashed.

All colorimetric determinations were made with a photo-electric colorimeter of the test-tube type, using uniform tubes calibrated at 5 and 10 ml.

Iron was determined in the dissolved ash by a slight modification of the A.O.A.C. colorimetric method (**2**). A 5-ml. aliquot of the dissolved ash was transferred to a colorimeter tube and 0.5 ml. of 20 per cent. KCNS added. The tube was shaken and a reading taken immediately in the colorimeter using a green filter (Wratten #54). One division on the colorimeter scale was equivalent to about 0.1 microgram of iron per 5 ml. of test solution and Beer's law was followed to a reading of about 400. In all iron determinations special care was taken to prevent contamination of the sample with iron. The solutions were never allowed to come in contact with cork or rubber and all leaf samples were thoroughly washed before being dried or frozen. A frequent blank reading on all reagents was taken.

Calcium was determined by a modified volumetric micro-method (**12**). A 5-ml. aliquot of the dissolved ash was transferred to a wide-mouth 250-ml. Erlenmeyer flask. Five ml. of a saturated solution of ammonium oxalate was added, followed by 5 ml. of a saturated solution of sodium acetate. After the solution had stood overnight, the supernatant liquid was removed by means of a sintered-glass filter-stick with a layer of asbestos over the tip. The precipitate was thoroughly washed with a saturated solution of calcium oxalate made alkaline with a few drops of ammonium hydroxide. The pad and the precipitate were washed into a flask with distilled water. One ml.

of 6 N sulphuric acid was added and the solution was boiled to dissolve the calcium oxalate. After cooling, an excess of 0.01 N ceric sulphate was added and the solution was titrated with 0.01 N ferrous ammonium sulphate, using o-phenanthroline for an indicator.

Potassium was determined by a modified micro-volumetric method in the initial work, but this procedure was later discarded for a more rapid turbidimetric method, which has been described elsewhere (**11**). In the volumetric method a 5-ml. aliquot of the dissolved ash was transferred to a 250-ml. wide-mouth Erlenmeyer flask. One ml. of 6 N hydrochloric acid was added, followed by 2.5 ml. of glycerine and 8 ml. of KRAMER and TISDALL'S cobaltinitrite reagent (**10**). The mixture was allowed to stand overnight and the supernatant liquid was removed from the precipitate by means of a sintered-glass filter-stick with a layer of asbestos and celite over the tip. The precipitate and flask were washed at least 3 times with 70 per cent. alcohol and the wash liquid was continually removed with the filter-stick. The precipitate and pad of asbestos and celite were washed into the flask with distilled water and brought to a volume of about 30 ml. The mixture was boiled until the precipitate dissolved. After cooling, an excess of 0.01 N ceric sulphate was added and the solution titrated with 0.01 N ferrous ammonium sulphate using o-phenanthroline as an indicator.

Magnesium and phosphorus were determined by colorimetric methods that have been described elsewhere (**11**).

Manganese was determined by a slightly modified colorimetric A.O.A.C. method (**2**). A 5-ml. aliquot of the dissolved ash was transferred to a colorimeter tube. About 0.1 gm. of KIO_4 was added and the tube heated in a boiling water bath until maximum color had developed (at least 30 minutes). After the solution had been cooled and made back to the original volume with water, a reading was taken on the colorimeter using a green filter (Wratten #54). One division on the colorimeter scale was equivalent to about 0.25 microgram of manganese per 5 ml. of test solution. BEER'S law was followed up to a reading of about 400.

Chlorophyll was determined on fresh or frozen material by a slightly modified procedure of PETERING, WOLMAN, and HIBBARD (**23**). A red filter was used in the colorimeter (Wratten #29, with two pieces of ground glass to reduce light intensity). An ether solution of the chlorophyll was transferred to a colorimeter tube which was stoppered to prevent evaporation. The colorimeter was standardized against 5 X chlorophyll obtained from American Chlorophyll, Incorporated. Since BEER'S law was not followed exactly, a standard curve had to be prepared. In the lower ranges, 1 division on the colorimeter scale was equivalent to about 0.7 microgram of chlorophyll per 5 ml. of test solution.

All analyses reported in this paper are the average of duplicate or triplicate determinations.

FRACTIONATION PROCEDURES

Leaf samples of 100 to 200 sq. cm. in area (1 to 2 gm. dry wt.) were extracted with various solvents in the following manner:

The area of the leaves was obtained by tracing their outlines on paper and subsequently measuring the area with a planimeter. The leaf samples were then carefully washed to remove all adhering dust, placed in clean cellophane sacks, and frozen. Each sample, after it had been thawed, was ground in an appropriate-sized porcelain mortar. About 30 ml. of 50 per cent. acetone was added and the extract decanted through a piece of Whatman #41H filter paper on a 7-cm. Buchner funnel. The sample was further extracted several times with 50 per cent. acetone until a total of 100 ml. had been used. The grinding and extraction were continued, with pure acetone as a solvent, until the leaf tissue was reduced to a fine white or brownish powder. A total of 100 ml. of pure acetone was used in this later extraction. The filtrate was transferred to a separatory funnel and about 70 ml. of ether was added. The solution was mixed and allowed to stand several minutes to permit the ether layer to separate. The water layer was transferred to a 250-ml. beaker. The ether layer, containing the chlorophyll, was washed several times with water, and each of the washings was added to the water fraction in the beaker. The ether fraction was then transferred to a glass-stoppered graduated cylinder and made to 100-ml. volume with ether. Chlorophyll was determined on this fraction by the method previously indicated. In the text the ether extract of the water-acetone fraction is designated as the "ether" fraction.

The leaf residue from this extraction was treated with about 50 ml. of 0.1 N HCl and transferred to the Buchner funnel along with washings of 0.1 N HCl. The extraction was continued until 150 ml. of HCl had been used. The filtrate was transferred to a 250-ml. beaker and the residue was successively extracted in the same manner with 0.5 N HCl and then with 1 N HCl. The final residue, including the filter paper, was then transferred along with a little wash water to a 250-ml. beaker. One ml. of the sodium hydroxide–sodium nitrate ashing reagent was added to each extract and 5 ml. to the residue. All extracts were evaporated to dryness, and ashed at 450° C. in a muffle furnace as indicated previously.

In some cases (particularly with pear leaves) filtration was so extremely slow that it was necessary to separate the extract and residue by centrifuging. Both methods gave similar results.

By extracting first with 50 per cent. and then 100 per cent. acetone, it was found that a more rapid extraction was possible than by making a water extract, and the value of the water extract was still retained by separating the fat-soluble material with ether.

OSERKOWSKY's extraction procedure (**21**) differed considerably from that given above. He used a larger initial sample (5 gm.) and extracted it with about 50 ml. of 1 N HCl by shaking for a definite period of time. After centrifuging, he washed the residue with an additional 50 ml. of acid.

In comparing OSERKOWSKY's method with the one given here, it was found that fresh or frozen samples could be used as well as dry samples, but when cholorphyll was to be determined, it was necessary to use only fresh

or frozen material. Furthermore, repeated tests showed that practically all soluble material was removed with the first 50 ml. of solvent when a small (1 to 2 gm. dry wt.) sample was used. By using 150 ml. of solvent with this size of sample, a fairly complete extraction was assured.

Results

In preliminary work, an attempt was made to find by electrodialysis the iron fraction in leaves that was active in chlorophyll formation. OSERKOWSKY's results (**21**) indicated that only a small part of the iron in the leaf was active in chlorophyll formation, and that in leaves affected with lime-induced chlorosis, the iron was in an inactive form.

Fresh and frozen samples were suspended in distilled water between platinum electrodes 3 sq. cm. in area and about 3.5 cm. apart. Varying

TABLE I

IRON EXTRACTED FROM NORMAL AND CHLOROTIC BARTLETT PEAR LEAVES BY ELECTRODIALYSIS AT 180 VOLTS FOR 2 HOURS. ELECTRODES 3 SQ. CM. IN AREA AND 3.5 CM. APART. SAMPLES COLLECTED ON 7/16/40

SAMPLE	FRACTION	IRON PER 100 SQ. CM. OF LEAF AREA
		γ
Green leaves from chlorotic Bartlett tree	Dialysate	3
	Residue	84
	Total	87
Chlorotic leaves from same tree as above	Dialysate	5
	Residue	47
	Total	52
Leaves from chlorotic Bartlett pear tree that turned green after iron citrate was injected into the tree on 5/21/40	Dialysate	17
	Residue	156
	Total	173

voltages were tried for various lengths of time, but in no case could the amount of iron removed be correlated with the chlorophyll content of the leaves. Differences between fresh and frozen samples were usually not very great although more iron was commonly extracted from frozen samples. Table I shows some data typical of those obtained.

It was concluded from this work that the dialyzable iron was not correlated with lime-induced chlorosis, and that the iron active in chlorophyll formation was probably present in some complex insoluble form. An attempt was then made to extract the "active" iron from the leaf by some means other than that used by OSERKOWSKY (**21**). Green and chlorotic leaves were extracted with water, alcohol, acetone, 1 N acetic acid, and 1 N ammonium hydroxide, but the iron content of these extracts could not be correlated with chlorosis.

CALCIUM/POTASSIUM RELATIONS IN LIME-INDUCED CHLOROSIS

In order to determine whether some of the more common elements other than iron might be correlated with lime-induced chlorosis, green and chlo-

TABLE II

IRON, CALCIUM, AND POTASSIUM CONTENT* OF DELICIOUS APPLE LEAVES IN RELATION TO CHLOROSIS. SAMPLES TAKEN FROM PARTIALLY CHLOROTIC TREE GROWING IN CALCAREOUS SOIL ON 8/2/40

SAMPLE	Fe	Ca	K
	p.p.m.	%	%
Green leaves	188	1.90	1.13
Slightly chlorotic	205	2.36	2.71
Moderately chlorotic	173	1.78	4.49
Severely chlorotic	159	1.37	5.18

* Calculated on dry weight basis.

rotic leaves were analyzed for total nitrogen, phosphorus, calcium, potassium, and iron. There seemed to be no direct relation between the phosphorus and the nitrogen content and the degree of chlorosis. However, a striking degree of correlation between the amount of chlorosis and the potassium content was found (tables II, III).

A number of similar determinations were made on different kinds of plants and some typical results are presented in table IV. There was no consistent correlation between the total iron content and the degree of chlorosis but chlorotic leaves were always found to be considerably higher in potassium.

The higher calcium/potassium ratio in green than in chlorotic leaves raised the question whether the relationship between calcium and potassium in chlorotic leaves was modified when the leaves became green following iron citrate injection into the tree. Two severely chlorotic Bartlett pear trees growing on calcareous soil, one limb on each of which had been injected with iron citrate on May 21, 1940, furnished material for this purpose. The leaves on the treated limb on one tree became fully green, while on the other tree the leaves became only partially green. The results of the analyses are shown in table V. The injection of iron citrate did not reduce the potassium content of the initially chlorotic leaves to that of the normal green leaves, but the calcium content was greatly increased and, as a result, the calcium/potassium relation was restored to nearly that of normal green leaves.

TABLE III

IRON, CALCIUM, POTASSIUM, AND CHLOROPHYLL CONTENT OF BARTLETT PEAR LEAVES IN RELATION TO CHLOROSIS. SAMPLES TAKEN FROM PARTIALLY CHLOROTIC TREE GROWING IN CALCAREOUS SOIL ON 8/27/40

SAMPLE	Fe*	Ca*	K*	CHLOROPHYLL†
	p.p.m.	%	%	%
Green leaves	158	2.54	1.11	0.31
Slightly chlorotic	148	2.79	1.15	0.20
Moderately chlorotic	79	2.53	2.47	0.07
Severely chlorotic	43	2.97	3.55	0.03

* Calculated on dry weight basis.
† Calculated on fresh weight basis.

TABLE IV

IRON, CALCIUM, AND POTASSIUM CONTENT* OF PAIRED SAMPLES OF GREEN AND CHLOROTIC LEAVES FROM PARTIALLY CHLOROTIC PLANTS GROWING IN CALCAREOUS SOIL

PLANT	DATE	SAMPLE	Fe	Ca	K	Ca/K
			p.p.m.	%	%	
Bartlett pear	7/16/40	Green	87	2.43	0.54	4.50
		Severely chlorotic	52	1.13	1.90	0.59
Riland apricot	8/22/40	Green	86	1.70	3.20	0.53
		Severely chlorotic	326	1.86	5.05	0.37
Wild rose	8/27/40	Slightly chlorotic	155	1.53	1.54	0.99
		Severely chlorotic	318	1.87	2.29	0.82
Delicious apple	8/27/40	Green	183	1.22	1.51	0.81
		Severely chlorotic	83	0.89	2.89	0.31
Delicious apple	8/28/40	Green	40	1.02	1.98	0.52
		Moderately chlorotic	29	1.36	2.77	0.41
Slappy peach	8/31/40	Green	98	2.21	1.46	1.51
		Severely chlorotic	83	1.42	2.52	0.56
Bing cherry	9/27/40	Green	66	4.04	1.57	2.57
		Severely chlorotic	68	3.92	2.99	1.31

* Calculated on dry weight basis.

It is not known whether the calcium/potassium relations are the cause or the result of lime-induced chlorosis, but there seems to be a consistent correlation. The high level of potassium in chlorotic leaves is probably responsible for the burning that is often observed when chlorosis is severe.

LEAF FRACTIONATIONS IN LIME-INDUCED CHLOROSIS

In order to clarify these nutrient interrelations, an attempt was made to separate the various chemical elements into different fractions by successively extracting green and chlorotic leaves with a series of solvents. By means of this procedure it was hoped that some idea might be obtained as to the approximate types of compounds of the various elements that are present in both green and chlorotic leaves.

TABLE V

IRON, CALCIUM, AND POTASSIUM CONTENT* OF PAIRED SAMPLES OF BARTLETT PEAR LEAVES AS AFFECTED BY INJECTING IRON CITRATE INTO THE TREE. TREES INJECTED 5/21/40

SAMPLE	DATE	Fe	Ca	K	Ca/K
		p.p.m.	%	%	
Tree no. 1					
Chlorotic leaves from untreated limb	7/16/40	28	1.88	2.35	0.80
Leaves fully greened by injection	7/16/40	173	3.02	2.10	1.44
Tree no. 2					
Chlorotic leaves from untreated limb	7/25/40	31	1.51	1.85	0.82
Leaves partially greened by injection	7/25/40	72	3.22	2.03	1.59
Average normal pear leaves for comparison	7/25/40	95	2.64	.99	2.67

* Calculated on dry weight basis.

TABLE VI

IRON FRACTIONS IN PAIRED SAMPLES OF NORMAL AND CHLOROTIC BARTLETT PEAR LEAVES TAKEN FROM THE SAME TREE IN JUNE AND AUGUST COMPARED WITH CHLOROPHYLL CONTENT

FRACTION	Fe IN MICROGRAMS PER 100 SQ. CM. OF LEAF AREA			
	6/23/41		8/12/41	
	GREEN	CHLOROTIC	GREEN	CHLOROTIC
	γ	γ	γ	γ
Water	4	3	4	21
"Ether"	1	1	2	2
0.1 N HCl	14	15	20	9
0.5 N HCl	15	7	24	11
1.0 N HCl	7	7	15	4
Insoluble	28	44	68	80
Total	69	77	133	127
Chlorophyll in mg. per 100 sq. cm.	1.72	0.21	5.10	0.40

Using the previously described extraction procedure, samples of green and chlorotic Bartlett pear leaves, collected in June and August, were analyzed for iron, calcium, potassium, phosphorus, manganese, and magnesium. The data on iron are summarized in table VI. The chlorotic samples were from a very severely chlorotic tree while the green samples were from a normal appearing adjacent tree, both growing in calcareous soil. In June, only the 0.5 N acid extract showed lower in iron for chlorotic leaves than for green ones, while the water-soluble fraction was much higher.

OSERKOWSKY (**21**) obtained more iron in his 1 N acid fraction of chlorotic leaves later in the season than he did from green leaves, while early in the season he obtained more iron in this fraction in the green leaves. This might have been due to the presence of more readily soluble iron in his samples during the latter part of the growing season.

TABLE VII

CALCIUM, POTASSIUM, MAGNESIUM, PHOSPHORUS, AND MANGANESE FRACTIONS* IN GREEN AND CHLOROTIC BARTLETT PEAR LEAVES IN AUGUST

FRACTION	Ca		K		Mg		P		Mn	
	G†	C‡	G	C	G	C	G	C	G	C
	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ
Water	2100	3600	5500	14200	2700	3500	270	420	16	28
"Ether"	100	0	100	400	100	0	40	50	0	0
0.1 N HCl	11100	7700	300	3100	3200	1100	280	200	27	19
0.5 N HCl	9700	6300	0	100	100	200	60	40	2	0
1.0 N HCl	3000	1200	0	0	0	0	30	30	0	3
Insoluble	800	100	0	700	700	0	540	500	0	0
Total	26800	18900	5900	18500	6800	4800	1220	1240	45	50

* Micrograms per 100 sq. cm.
† G = green.
‡ C = chlorotic.

TABLE VIII

IRON, CALCIUM, POTASSIUM, MAGNESIUM, PHOSPHORUS, AND MANGANESE FRACTIONS* IN CHLOROTIC BARTLETT PEAR LEAVES AND LEAVES FROM THE SAME TREE BUT ON THE SIDE THAT HAD GREENED AFTER SOIL TREATMENT WITH IRON SULPHATE

FRACTION	Fe		Ca		K		Mg		P		Mn	
	G†	C‡	G	C	G	C	G	C	G	C	G	C
	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ
Water	4	4	1450	1240	8600	13600	1470	2270	130	380	22	10
"Ether"	8	2	0	0	900	1100	60	0	70	40	0	0
0.1 N HCl	17	3	9140	5040	3400	7600	1610	640	170	430	66	21
0.5 N HCl	25	6	6270	7370	1000	0	160	80	70	370	0	0
1.0 N HCl	17	2	4610	430	0	0	0	0	40	80	0	0
Insoluble	23	18	1450	0	700	0	60	0	380	580	0	0
Total	94	35	22920	14080	14600	22300	3360	2990	860	1880	88	31

* Micrograms per 100 sq. cm.
† G = green.
‡ C = chlorotic.

From the data in table VI it might be assumed that the 0.5 N acid fraction contained the iron associated with chlorophyll formation. The total iron in this fraction, however, was not directly proportional to the chlorophyll content.

The water-soluble fraction in both green and chlorotic leaves was found to be low in iron, as was found to be the case by electrodialysis (table I). About half the total iron in the leaf was in a form insoluble in the solvents used.

In table VII the results of other analyses are presented. There was much more potassium in all fractions of the chlorotic leaves than in corresponding fractions of the green leaves, with the exception of the 1 N acid fraction which did not yield any potassium even from chlorotic leaves. The chlorotic condition increased the water-soluble form of all other elements. The chlorotic leaves again show a somewhat lower total calcium content than do the

TABLE IX

IRON, CALCIUM, POTASSIUM, MAGNESIUM, PHOSPHORUS, AND MANGANESE FRACTIONS* IN PREVIOUSLY CHLOROTIC BARTLETT PEAR LEAVES THAT WERE GREENED BY THE TREATMENT OF LEAVES LOWER DOWN ON THE SAME BRANCH WITH IRON CITRATE

FRACTION	Fe	Ca	K	Mg	P	Mn
	γ	γ	γ	γ	γ	γ
Water	6	2000	17700	2900	250	18
"Ether"	2	0	0	100	50	0
0.1 N HCl	40	8900	1900	1100	170	32
0.5 N HCl	55	8200	0	0	70	0
1.0 N HCl	24	5900	0	0	30	0
Insoluble	71	800	0	0	560	0
Total	198	25800	19600	4100	1130	50

* Micrograms per 100 sq. cm.

green leaves. Total magnesium, likewise, is somewhat lower in chlorotic leaves, while phosphorus and manganese seem to be essentially the same in both green and chlorotic leaves. It is of interest to note that manganese seems to be present in forms which are either water soluble or easily dissociated. It must form compounds in the plant which are quite different from those of iron.

In order to determine whether the 0.5 N acid fraction had increased in iron when chlorotic leaves had been greened by a soil treatment with iron sulphate, green and chlorotic leaf samples were taken from a Bartlett pear tree in August. One side of the tree had been given a soil treatment by trenching in iron sulphate in May. Only the treated side of the tree had become green, and thus it was possible to obtain both green and chlorotic samples from the same tree. The results of the analyses of these samples are shown in table VIII. In the green leaves all fractions, except the water-soluble fraction, were higher in iron than in the chlorotic leaves. The total amounts of calcium, magnesium, manganese, and iron were higher in the green leaves, while the total amounts of potassium and phosphorus were

lower. Here again the calcium/potassium relation appeared to be associated with chlorosis.

Somewhat similar effects were obtained when chlorotic leaves were greened by dipping them in solutions of iron salts. The solutions were strong enough to burn the leaves that were dipped, but enough iron was absorbed and translocated to other leaves on the same branch to cause them to turn green. In table IX are presented the results obtained from the analyses of leaves that had become green after leaves lower down on the same branch had been dipped in iron citrate solution. The dipping was performed on May 23, 1941, and leaf samples were taken on August 23, 1941. These data may be compared with the August samples in the two previous tables (VII, VIII). The chlorophyll content of this sample was 2.20 mg. per 100 sq. cm. Here again the calcium and the iron content were markedly greater than in chlorotic leaves. The 0.5 N acid fraction was relatively high in iron. The total potassium content, however, was not markedly lower.

Leaf fractionations in true iron deficiency

It became of interest to determine whether these nutrient relations would be found in the case of true iron deficiency as well as in lime-induced chlorosis. Apple seedlings were grown for three months in the laboratory in water culture with and without added iron. Leaves on the plants growing in the solutions from which the iron was withheld soon turned quite yellow. These yellow leaves, and comparable leaves from green plants, were fractionated as in the previous tests. Table X shows the results of these analyses. Since the chlorotic leaves were only about one-half the size of the green leaves, the results were expressed on a per leaf basis as well as on an area basis. The relative distribution of the elements in the various fractions should be considered here rather than the absolute amounts in these fractions. The area basis should probably not be used because of the difference in leaf size. In all previous samples the normal and chlorotic leaves were of about the same size.

As seen in table X, the calcium/potassium relations found to prevail in lime-induced chlorosis do not seem to hold for true iron deficiency. The iron is low in all the acid soluble fractions in the chlorotic leaves, including the 0.5 N acid fraction. It is also interesting to note that the water soluble iron is very low in the chlorotic leaves. The chlorophyll content was 79 micrograms per leaf in the yellow leaves and 362 micrograms in the green leaves. The differences in chlorophyll content are much greater than any differences in iron content.

Leaf fractionations in genetically yellow Spiraea

The question then arose as to whether genetically variegated leaves had the same nutrient relations as leaves affected with lime-induced chlorosis. A comparison was made of fully green and completely yellow Spiraea sp. leaves from the same plant. The data are shown in table XI. In the case

TABLE X

IRON, CALCIUM, POTASSIUM, MAGNESIUM, PHOSPHORUS, AND MANGANESE FRACTIONS IN GREEN AND CHLOROTIC LEAVES OF APPLE SEEDLINGS GROWING IN WATER CULTURES WITH AND WITHOUT ADDED IRON

FRACTION	Fe		Ca		K		Mg		P		Mn	
	G*	C†	G	C	G	C	G	C	G	C	G	C
	MICROGRAMS PER 100 SQ. CM.											
Water	20	1	670	920	8320	8410	610	1310	260	550	20	38
"Ether"	11	32	20	0	140	880	130	0	40	80	0	0
0.1 N HCl	22	20	1580	2730	1330	440	410	340	10	60	25	28
0.5 N HCl	11	10	310	0	380	880	0	0	0	20	0	0
1.0 N HCl	9	11	60	0	0	660	0	0	10	0	0	0
Insoluble	12	49	50	0	0	0	0	0	130	240	0	0
Total	85	123	2690	3650	10170	11270	1150	1650	450	950	45	66
	MICROGRAMS PER LEAF											
Water	2.7	0.1	91	57	1137	518	84	81	36	34	2.8	2.4
"Ether"	1.6	2.0	2	0	19	54	18	0	5	5	0.0	0.0
0.1 N HCl	3.0	1.2	217	169	182	27	56	21	1	4	3.4	1.5
0.5 N HCl	1.3	0.6	42	0	52	54	0	0	0	1	0.0	0.0
1.0 N HCl	1.2	0.7	9	0	0	40	0	0	1	0	0.0	0.0
Insoluble	1.7	3.0	7	0	0	0	0	0	17	15	0.0	0.0
Total	11.5	7.6	368	226	1390	693	158	102	60	59	6.2	3.9

* G = green (iron added).
† C = chlorotic (iron not added).

TABLE XI

IRON, CALCIUM, POTASSIUM, MAGNESIUM, PHOSPHORUS, AND MANGANESE FRACTIONS* OF GREEN AND GENETICALLY YELLOW SPIRAEA LEAVES

FRACTION	Fe		Ca		K		Mg		P		Mn	
	G†	Y‡	G	Y	G	Y	G	Y	G	Y	G	Y
	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ
Water	11	21	370	100	7490	11550	103	551	737	939	3	10
"Ether"	20	18	0	0	0	0	78	0	94	44	0	0
0.1 N HCl	18	16	1290	560	0	1520	514	388	43	44	11	10
0.5 N HCl	14	14	0	40	0	0	103	0	35	21	0	0
1.0 N HCl	14	15	0	20	0	0	0	0	43	33	0	0
Insoluble	28	45	0	70	0	0	154	0	343	248	0	0
Total	105	129	1660	790	7490	13070	952	939	1295	1329	14	20

* Micrograms per 100 sq. cm.
† G = green.
‡ Y = genetically yellow.

of lime-induced chlorosis, leaves which appeared almost white were found to contain small amounts of chlorophyll. The yellow Spiraea leaves, however, were completely devoid of chlorophyll, a fact which indicated some basic upset in chlorophyll manufacture. The green leaves had 3.85 mg. of chlorophyll per 100 sq. cm. There was less calcium in the yellow leaves but more potassium. This condition is much like that of lime-induced chlorosis. The 0.5 N acid fraction, however, was not low in iron in the yellow leaves, indicating that some factor other than iron is probably responsible for the lack of chlorophyll formation in these leaves.

Discussion

In the work reported here, no consistent correlation was found between the total iron content of the leaves and lime-induced chlorosis. MILAD (**16**), AILLYN (**1**), WALLACE (**32**), and OSERKOWSKY (**21**) have all reported similar results. Furthermore, there seemed to be no correlation between lime-induced chlorosis and either the phosphate/iron balance or the manganese/iron balance. It would thus appear that lime-induced chlorosis is not associated with these other types of iron chlorosis. In some cases, a somewhat lower iron content may be a contributing factor in lime-induced chlorosis, but other factors must also be in play. The data presented here may be criticized in that samples were usually taken during June, July, and August instead of earlier in the season when chloroplasts are thought to be more active. However, under our conditions solutions of iron salts applied to chlorotic foliage as late as mid-July will show a greening effect on the leaves by late August, indicating that the chloroplasts are still able to synthesize chlorophyll during this period.

Iron undoubtedly forms many different compounds within the tissues of the plant. Long ago, MACALLUM (**13**) demonstrated that there existed firmly combined iron in plant tissue, particularly associated with nuclei. More recently, REED and DUFRENOY (**24**) demonstrated that in orange leaves iron was mainly associated with the plastids and nuclei. NOACK and LIEBICH (**18**) reported that 82 per cent. of the total iron in spinach leaves was associated with the chloroplasts. They separated this iron into various fractions, and concluded that five-sixths of the firmly bound iron was absorbed on phosphorus-containing proteins. THEORELL (**30**) recently summarized the known iron porphyrin enzymes and listed them as: (a), Warburg's respiratory ferment; (b), the cytochromes—a, b, and c; (c), the catalases; and (d), the peroxidases. SAYRE (**26**) studied the iron deposits in corn stems and concluded that they were complex hydrous iron oxides combined with proteins.

These several investigations, in addition to the fractionation results reported in this paper, suggest that in the iron nutrition of a plant there is a competition between the various compounds in the plant for the available iron. There is probably a dynamic equilibrium in leaf tissue between such forms of iron as iron ions, iron salts of organic acids, iron-phosphate com-

plexes, iron-hydroxide complexes, iron-silica complexes, lipoidal iron, hematin iron, iron-nucleoproteins, and possibly the iron-containing "chlorophyll enzyme." In addition, there probably is an equilibrium between the two oxidation states of iron. When the nutrition of the plant is upset in any way, there could easily be a shift in the equilibrium between the various iron compounds.

Even though there seems to be many iron compounds in the plant, there are, as yet, only two known functions of iron. One of these functions is as a constituent of many enzymes that take part in the respiratory system. The other rôle appears to be as a catalyst in chlorophyll formation. This iron-containing "chlorophyll enzyme" seems to be one of the least stable of the iron compounds, at least its deficiency is one of the most readily observed results of an upset iron metabolism. It is quite probable that some or all of the other iron compounds play certain specific rôles in plant nutrition, but their functions have not as yet been ascertained.

One difficulty in working with an element, such as iron, that is present in very low concentrations in plant material is that the methods of analysis may not be sensitive enough to detect certain very important compounds containing the element. It is quite conceivable that an enzyme may be present in low enough concentration in plant material to allow for less than 1 p.p.m. of iron, for 1 p.p.m. of iron is about equal to 1×10^{16} molecules per gram of leaf tissue.

When iron was withheld from apple seedlings in water culture, the water-soluble fraction from the leaves of these plants was low in iron, a fact which would seem to indicate that substances in this fraction are not able to compete with those of other fractions for available iron. The 0.5 N acid fraction is also low under conditions of true iron deficiency, and it is also consistently low in the case of lime-induced chlorosis. This suggests that 0.5 N HCl removes the iron from the enzyme that is responsible for chlorophyll formation. The iron content of this fraction is not directly proportional to the chlorophyll content of the leaf. This might be expected, however, because iron other than that of the chlorophyll enzyme could very well be present in this fraction. Furthermore, the rate of enzyme action is not necessarily directly proportional to the amount of enzyme present; even though this fraction contained only that iron present in the chlorophyll enzyme, it would not necessarily follow that the iron content would be directly proportional to the chlorophyll content.

A high potassium content was also correlated with lime-induced chlorosis. It would appear that the high potassium content of chlorotic leaves caused the iron to be displaced from the enzyme responsible for chlorophyll formation, resulting in the inactivation of this enzyme. However, further proof is needed for this hypothesis. In this regard, WALSH and CLARKE (**33**) have recently reported a chlorosis of tomato plants associated with a luxury consumption of potassium.

The magnesium content was found to be low in the "ether" fraction of

chlorotic leaves, but this was probably due to the fact that the chlorophyll content was low rather than to a derangement in magnesium metabolism.

The calcium/potassium relations of lime-induced chlorosis have been pointed out before by other workers, but no great significance has been attached to them. As long ago as 1886, CHURCH (**4**) reported that chlorotic oak leaves were high in potassium and low in calcium. COLIN and GRANDSIRE (**6**) have shown the same relation in chlorotic leaves of chestnuts and elms and WALLACE and MANN (**31**) for apple. In later work, WALLACE (**32**) has shown the same relations for lime-induced chlorosis of apple, pear, plum, and raspberry. GRANDSIRE (**7**) has also reported a high potassium and low calcium content of albino leaves. The fact that genetic albinism induces an upset in calcium/potassium relations does not necessarily prove that these relations are not connected with the cause of lime-induced chlorosis. The work reported here, and the work of OLSEN (**19**), indicates that chlorosis brought about by true iron deficiency or by an upset in phosphate/iron balance is not associated with an upset in calcium/potassium relations. Thus leaves may be low in chlorophyll and still not accumulate large amounts of potassium. Furthermore, MCGEORGE (**14**) has shown that plants growing in calcareous soil pick up very large amounts of potassium. Thus it is conceivable that lime-induced chlorosis may be brought about in part by a high potassium level of the leaves. In any nutritional explanation of this type of chlorosis, it will be necessary to take into account the fact that only parts of a tree may be chlorotic and that even adjacent leaves may vary markedly in their degree of chlorosis.

CHAPMAN (**3**) and HAAS (**8**) have shown that the maintenance of a high moisture level in calcareous soils tended to increase the incidence of chlorosis, while a low moisture level tended to reduce it. According to their explanation, an increased water supply increased the hydrolysis of calcium carbonate and resulted in an increase of hydroxyl ion concentration. However, PARSCHE (**22**) has shown that calcium chloride, as well as calcium carbonate, may induce iron chlorosis of lupine, even though the calcium chloride did not affect the pH of the soil appreciably. Thus it is possible that soil pH plays a rôle in lime-induced chlorosis only insofar as it affects the calcium/potassium relations. This is further indicated in the work of HOFFER (**9**) and others who have shown that corn plants deficient in potassium accumulate large amounts of iron.

Lime-induced chlorosis is a complex disorder and needs more investigation before much light can be thrown on its physiology. Further experiments are in progress and it is hoped they will help elucidate some of the problems discussed here.

Summary

1. Lime-induced chlorosis of plants is distinguished from other types of iron chlorosis such as true iron deficiency, upset manganese/iron balance, and upset phosphate/iron balance.

2. Leaves affected with lime-induced chlorosis are high in potassium but

somewhat low in calcium and magnesium. It is not definitely established whether these nutrient relations are associated with the cause or are a result of chlorosis.

3. Data are presented on the distribution of Ca, K, Mg, P, Fe, and Mn in various fractions of green and chlorotic pear and apple leaves and of green and variegated Spiraea leaves. The potassium was found to be mostly water-soluble. Magnesium and manganese were either water-soluble or readily dissociated by 0.1 N HCl (except for the small amount of magnesium present in the ether fraction as part of the chlorophyll molecule). Calcium, phosphorus, and iron, on the other hand, were distributed more generally in all of the fractions.

4. Over half the iron in the leaves examined was present in a form insoluble in 1 N HCl; presumably as iron-hematin, iron-nucleoprotein, or in other complex organic compounds.

5. Neither the total iron, insoluble iron, nor the iron soluble in water, ether, alcohol, 1 N acetic acid, 1 N ammonium hydroxide, 0.1 N HCl, or 1 N HCl was found to be correlated with lime-induced chlorosis. Likewise, electro-dialyzable iron could not be correlated with chlorosis.

6. The iron extracted by 0.5 N HCl was found to be low in chlorotic leaves. The 0.5 N HCl probably removed the iron from an enzyme that plays a rôle in chlorophyll formation.

7. Lime-induced chlorosis is probably brought about by a complex of causes whose interrelations are not yet fully established. The data suggest that a relatively high potassium level induces chlorosis by replacing the iron on the enzyme responsible for chlorophyll formation, thereby inactivating the enzyme.

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MYCORRHIZAL SYMBIOSIS IN APLECTRUM, CORALLORHIZA AND PINUS

D. T. MacDougal and J. Dufrenoy

(WITH NINE FIGURES)

Introduction

When plants of different types are closely crowded together or anatomically engaged, in addition to the ensuing competitions, several special relations including immunities, antagonisms, toxicities, parasitism, or symbiosis may arise.

A secretion by one plant which inhibits the growth of a neighboring organism, such as the substance found in the tissues of orchids by Noel Bernard which limits the growth of the hyphae of endophytic fungi, results in an immunity. Similar antagonisms exist between some fungi growing together in the soil in which a secretion from one limits the growth of the other (**11**).

This constructively defense mechanism varies widely. In one instance described by Stoller (**21**) *Psalliota campestris* produces a diffusible oxidizing substance, acting as a quinone which protects the mushroom against microorganisms producing sulphides.

The action of the "humoral" secretion in such cases is to be distinguished from toxicity by the fact that in the first case the secretion simply blocks some of the processes of cell-metabolism but does not destroy the mechanism; in toxic action a theoretical secretion causes irreversible and fatal changes in the cytochemical set-up of living cells of the organism affected. In parasitism, in addition to possible anatomical and cytochemical damage, a fatal extraction of nutritive material may ensue. Further distinction between parasitism and symbiosis will be made in the following pages.

A much heightened interest in the bacteriostatic secretions of molds, of which penicillin is the most notable because of its sterilizing action in surgery, now prevails. This secretion specifically inhibits the growth of gram-positive bacteria but does not destroy them; secretions such as patulin exert similar action on both gram-positive and gram-negative bacteria [see also Burgess (**2**)].

Scope of the present paper

The senior author had previously made some studies of mycorrhizae of some heaths and orchids [MacDougal (**14, 15**); and MacDougal and Lloyd (**16**)] and when isolated segments of mycorrhizal pine roots which were capable of extended existence with a nutritive program identical with that of a chlorophylless plant were found, an arrangement for collaboration in the study of the implied symbiosis was made with Dr. Dufrenoy early in 1940. Grants in support of the research were awarded by the American Philosophical Society and some of the results have been published and other material is in press [MacDougal and Dufrenoy (**17, 18, 19**)].

The present paper presents additional results obtained by a study of the action of mycorrhizal roots of the pine, when included in an intact system with the tree, and when grown in isolated soil cultures. A re-examination of the endophytic mycorrhizae of two orchids, *Corallorhiza maculata,* and its near relative, *Aplectrum hyemale,* in a perspective of known facts as to auxins and vitamins has made available some cytochemical features of value in the estimation of the cytochemical relationships in the two types of mycorrhizae involved.

Morphogenesis of mycorrhizae

Endophytic mycorrhiza of Corallorhiza

No record of the seedling stages of *Corallorhiza maculata* are available,[1]

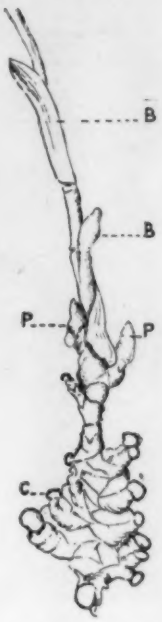


Fig. 1. Underground rootless stem of Corallorhiza with lower part of scape. C, coralloid branches. P,P, buds in autumnal condition; B,B, reddish bracts carrying traces of chlorophyll.

but the adult plant consists of a glistening white underground stem with irregularly placed thickened branches. No roots are to be seen. The older basal part of the axis dies away after the manner of a rhizome, which action may involve the branches as illustrated in figure 1. One or more scapes arise from the upper internodes. Foliar organs are represented by ap-

[1] Subsequent to the completion of the MS. of the present paper we have noted the record of observations by Dr. D. G. Downie (Trans. and Proc. Bot. Soc. Edinburgh 33: 380–382. 1943) on the symbiotic germination of seeds of *Corallorhiza innata* by association with a Basidiomycete. Later hyphae entered and formed characteristic coils in the basal cells of the slowly growing protocorms. Trichomes apparently similar to those of the coralloid branches of adult plants were developed.

pressed bracts in which a trace of chlorophyll and a few stomata present a capacity for transpiration and photosynthesis but little above zero.

The outer walls of some epidermal cells are extended into thick short trichomes suggestive of root-hairs. Hyphal branches pass outwardly through both types of epidermal cells, forming an absorptive system. The fungus develops dense swirls of hyphae surrounding the nuclei in the cortical cells. Generally the occupied cells show a random arrangement, but in a species collected by the senior author in Arizona two or three of the external layers not including the epidermis were filled; although the walls were intact a con-

FIG. 2. N, nucleus of cortical cell of Corallorhiza with portion of surrounding hyphae, H; stained blue with 2–6-dichloro-quinone-chloro-imide. ×715.

tinuous mycelium was formed [MACDOUGAL (**15**)]. In this case the hyphae invaded only the outermost half of the cortex, which is densely filled with starch and phenolic material.

Anatomically the whole arrangement is one by which soil-solutions may be absorbed by the external hyphae, elaborated, and translocated by the fungus (probably Rhizoctonia) to the cells of the higher plant. Functionally the metabolic possibilities are parallel to those offered by mycorrhizae of the pines.

Nuclei within the hyphal clumps were hypertrophied and granular, a modification which has been taken by many authors to imply a mechanism of exchange between the fungus and the orchid (fig. 2). This arrangement

presents some contrasts with that of pine mycorrhizae in which palmated hyphal branches entering the cortical cells engage finely vacuolated masses of cytoplasm near the nuclei. The disintegration of hyphal clumps in the cortical cells of the orchid takes place ahead of the death of the older tissues so that the material in the hyphae is liberated in living cells and thus becomes available to the higher plant conformable to the non-parasitic action of the fungus (fig. 5).

Reactions to tests for auxins demonstrated that some compounds of this kind were included in the substances yielded.

Cytochemical tests indicate that the hyphal branches in the outer part of the clumps are the site of a high indophenol (cytochrome) oxidase activity which may be a source of energy necessary for the transfer of material between the fungus and the higher plant (fig. 5).

Evolutionary development of mycorrhizae

Although some seed-plants upon which ectotrophic mycorrhizae are formed undergo great modifications of the shoot, yet roots are retained to be felted by the fungus partner. Plants in which the fungus partner inhabits the tissues of the higher form display much more advanced morphological alterations, inclusive of loss of roots and photosynthetic apparatus. Chlorophylless species to the number of over two hundred in the Burmanniaceae, Gentianaceae, Triuraceae, and Orchidaceae are included in this category. Many are reduced to small irregular underground stems and the scanty flower stalks which do not emerge from the heavy humus which they inhabit. [For example, see Degener (**4**)].

While some of the types exemplifying extreme reductions may have originated early in the history of the family to which they belong, it is not to be assumed that any correlation exists between the degree of modification and the antiquity of the adaptation; some of the alterations may have been of mutative occurrence, as exemplified by the occasional coralloid mycorrhiza of *Aplectrum hyemale* native to the northern United States and Canada.

Mutative origination of stem-mycorrhizae in Aplectrum

The greater number of plants of this species consist of an ovoid corm of three internodes attaining full size in late summer, at which time a single elongated, plaited, and ribbed leaf arises from an upper internode and persists through the winter. Early in the spring a scape arises from the axil of the leaf, bearing a loose raceme of flowers, and concurrently one or more offsets arise from a lower internode which reach a length of 2 to 4 cm. The terminals become apogeotropic and, turning upward while undergoing thickening, develop corms, thus effecting vegetative reproduction (fig. 3).

Twelve to twenty thick roots emerge from the base of each corm and attain a length of 5 to 10 cm. which bear a dense crop of large root-hairs extending from base to within 2 mm. of the root-cap, both root-hairs and cap being persistent. The outer walls of the epidermis and the walls of the root-

hairs soon become slightly suberized. The 4 to 8 layers of cortical cells contain dense clumps of hyphae of a fungus which has been identified by J. T. CURTIS (3) as *Rhizoctonia neottiae* Wolff. It is probable that the plants examined by MACDOUGAL contained a different species, as indicated by the lack of color of the hyphae.

FIG. 3. *Aplectrum hyemale* with normal form of mycorrhizal roots, and with mutative coralloid offset, *m*. *r*, abscission zone of leaf.

The hyphae traverse the cortex, and branches pass *out* into the soil through the root-hairs and epidermal cells in functional duplication of the arrangement in the underground *stems* of Corallorhiza.

The entrance of the fungus into the tissues of the individual plant is through a set of trichomes on the lower flank of the offset near the parent corm from which it originates. Here hyphal branches originating in the nearby roots, or from the soil, enter the trichomes and pass into the cortex of the offset, in which the growth of the hyphae follows the extension of the offset to its termination in a young corm with roots; progressive occupancy of the roots follows as described above.

Swirls of hyphae are formed in the lower quadrant of the cortex of the offset as the fungus extends to enter the roots of the young plant. No external branches are sent out through the epidermis of offsets in normal, or typical plants. The suggestion lies near that a secretion may be formed in the outer cortex and epidermis which inhibits growth of hyphal branches through these tissues, as outlined in the introduction to this paper, and as is known to occur in other parts of the plant.

It is in this stem-fungus association that the possibilities of a mutation lie; a mutation which has been seen to alter plants of Aplectrum so that they were adjusted to form coralloid or mycorrhizal stems anatomically similar to those of Corallorhiza, with which it is closely related [GRAY (**6**)]. That a parallel evolutionary path might be followed is supported by the reduction of the corms and foliar surfaces of plants which are formed at the terminals of coralloid offsets. Subsequent to the first record [MacDOUGAL (**14**)] plants with coralloid stems were obtained from Vermont and Minnesota (fig. 3).

ECTENDOTROPHIC MYCORRHIZA OF THE MONTEREY PINE

The seasonal activities of the root-system of the Monterey pine reflect the conditions of the climate, especially in the fact that elongation may begin during the winter season in which the soil temperature is not too low for growth. Apical growth of the terminals may be initiated upon repletion of the soil moisture at any time, beginning in November and December, and continue until the following summer with but haphazard connection with longitudinal growth of stems. Lateral short rootlets arise irregularly from initials in the pericycle and under favorable conditions become thickly mantled with hyphae of the symbiotic fungus before their final length of about 2 to 4 mm. is attained. Characteristic coralloid branching ensues and occasionally renewed growth exposes whitish tips.

The entire root system is in contact with the mycelia ramifying in the soil, hyphae of which mantle and penetrate the short roots only, the rapidly extending leaders and primary branches remaining free from investment. In seeking a theoretical cause of this differential action HATCH (**9**) records that while the main roots of *P. strobus* are diarch, the short roots which become mycorrhizal are monarch, lacking a root-cap, have only four layers of cortical cells, and reach their limited extension by slow growth. These features are seen in pure cultures and hence are not due to the action of the fungus. Short roots have been seen to originate in the pericycle and endodermis of roots of Monterey pine, but nothing can be said as to the inciting

agent. As will be discussed in a subsequent section, the perennity of the main roots is possible only by a type of respiration in which phosphorus linkages in the form of dehydrogenases is maintained in the apical meristem. Theoretically, the failure of this mechanism would account for the dwarfing and reduction of the short rootlets, but no supporting evidence is available.

In the external adjustment of the mycelial mantle to the rootlets the arrangement is similar to that of the ectotrophic mycorrhizae of the heaths. An additional feature of pine mycorrhizae is one by which branches of the hyphae which traverse the middle lamellae of cortical cells penetrate the inner walls of the cells in which the nuclei appear as large globular bodies with radiating strands of cytoplasm enclosing a network of anastomosed vacuoles, similar to the arrangements which have been described under the name of "the Golgi apparatus."

The arrangement in question is one which yields the maximum area of surface contact of vacuolar solution and cytoplasm, making possible a high rate of respiration and a high rate of translocation of material introduced by the fungus. It would appear that a similar arrangement was recognized by CHARLES DARWIN in the "aggregations" in the cells of the leaf-hairs of insectivorous plants, facilitating the translocation of introduced material in solution.

Each rootlet enters into a separate association with the investing fungus. Hyphae from the mycelial felt in the soil encounter the rootlets, not all of which may be so engaged. The fungus does not traverse the axis of the root from one branch to another; such longitudinal extension of the fungus in the tissues of the root is purely incidental, and generally takes place only at the end of the season when exfoliation of the cortex has begun. By reason of this manner of association, a root-system of a tree might be richly provided with mycorrhizae in one season, and devoid of them in the next by conditions in the humus unfavorable to the fungus partner.

This last-named condition has been brought about experimentally by the removal of seedlings, in the second season after some mycorrhizae had been formed, to water cultures in which the association does not occur.

The anatomical arrangement described justifies the designation of *ectendotrophic*, and it is one which is seen to furnish a highly efficient interchange between the two symbionts.

The coralloid branching supposedly resulting from the action of auxins produced by the hyphae on the endodermis and pericycle of the monarchic short roots are the only morphological effects of the association. The seasonal death of the mycorrhizae may leave the root system entirely free and a new crop of lateral rootlets are infected *de novo* by the hyphae ramifying through the soil.

Cyto-physiology of mycorrhizae

CYTOCHEMISTRY OF HYPHAE

No comprehensive analysis of the chain of metabolic processes in the hyphae of the fungal symbiont of the pines can be made, but a few of the

more important features can be detected. Free-hand sections of fresh material vitally stained with neutral red prepared in Ringer's solution or in reagents for oxidases were employed. Other results of importance were secured by killing mycorrhizal roots in Helly's fixative, imbedding in paraffin, sectioning with a microtome, then staining with haematoxylin, acid fuchsin, or molybdenum blue as decribed below.

In vital staining with neutral red in Ringer's solution the vacuolar solutions became pink-purplish, and red precipitates flocculated, a reaction taken to indicate an acid solution rich in polyphenols and presumably the site of a high cytochrome and polyphenolase activity.

In microtome sections of imbedded mycorrhizal roots stained with haematoxylin, acid fuchsin, or molybdenum blue the cytoplasmic meshwork enclosing the vacuoles are clearly defined; the mitochondria appear clearly as well as the several nuclei in each unit of the hyphae.

Cytoplasmic strands connecting the separate units through the central pores of the cross walls were seen. Pads of material at the pores, staining black by haematoxylin after fixation in Helly fluid (fig. 4), and red in acid

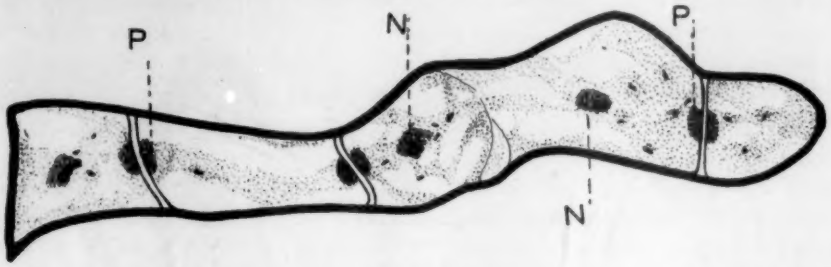


FIG. 4. Sketch of chain of cells of hyphae of pine mycorrhiza. N,N, nuclei with connecting mesh of cytoplasm. P,P, pads or cushions of material at pores in cross walls.

fuchsin and methyl green, negative their characterization as "optical illusions" by BULLER (1). These bodies from their histochemical character may be considered homologous to the callose drops at the sieve plates of the red algae, and to the sieve plates in the conducting elements in the phloem of seed-plants, *i.e.*, in elements through which translocation occurs.

The occurrence of auxins in the hyphae surrounding the nuclei in Corallorhiza and the release of this substance in mature cells is noted in a subsequent paragraph. The localization of oxidases in the outer part of the swirls of the nuclei has also been noted (fig. 5).

When sections of the heavy stems of Corallorhiza are fixed in Helly fluid and placed in a boiling solution of triketohydrindene hydrate, hyphae developed a purple color, while the nucleolus of the cortical cell was of a deeper tinge, with the body of the nucleus uncolored. The presence of proteids and amino acids was indicated.

IDENTIFICATION OF SITES OF OXIDASE ACTIVITY

The best fixative was found to be Helly's fluid, made up as follows: a stock solution of 25 gm. potassium bichromate and 50 gm. mercuric bichlo-

ride in one liter of distilled water was kept on hand. A mixture for treatment of pieces of tissue was made by adding 1 ml. neutralized 40 per cent. formaldehyde to 9 ml. of the stock solution. After twelve hours' immersion a brown sediment might develop, in which case the material was transferred to fresh solution of the same concentration for twelve hours. The sections were then placed in a 3 per cent. solution of potassium bichromate for a few days, then washed in running water for a few hours. Some of the results described were made by staining hand sections, and others by the paraffine method.

The mercuric chloride of the reagent was reduced in regions of high activity, with the result that the cytoplasm around vacuoles was marked by the presence of heavy black dots of mercuric precipitates. Another test was one in which hyphae in sections treated with paraphenylediamine and thymol was followed by an indophenol blue reaction, demonstrating oxidase activity.

This test gave marked positive reactions in the hyphal felt clothing coralloid roots, the color extending into the outermost layers of the cortex. The cytochrome oxidase indicated is present in the apical meristem of normal roots. The pericycle and endodermis are the site of phenoloxidases and lipidoxidases with the resultant production of tannins and of suberization, which constitute an effective barrier to the penetration of the stele by the fungus (figs. 5, 6).

Definite zonation of cytochemical features of the heavy stems of Corallorhiza does not exist. This, as will be noted later, is true of auxins as well as of the oxidases. Fresh sections treated with the reagent giving a nascent indophenol blue, stains some of the hyphae in the outer part of the swirl surrounding the nuclei a general blue, interpreted as a reaction of cytochrome oxidase, with some granules of deeper shade taken to be phospholipids. The reaction is seen only in certain cells supposedly in active growth.

Auxins in mycorrhizae

Much evidence is available to the effect that substances promoting growth in secondary meristem originate chiefly in leaves and buds, from which region they are translocated downward in the main axis as far as the root-system. This scheme is perhaps never strictly correct except under experimental conditions, as external agencies may induce intensive respiration and localized formation of these substances.

The continued growth of isolated segments of mycorrhizal roots would imply that these substances were obtained independently of the action of the stems or shoots.

Hitchcock and Zimmerman (10) found that normal roots might absorb from the soil and transmit to distant parts of the shoots added substances such as alpha-naphthaleneacetic acid, indole-acetic acid, indole-butyric acid, indole-propionic acid, phenylacetic acid, and the methyl and ethyl esters of these acids. In confirmation of the above results, seedlings grown aseptically in a nutrient solution containing 1 part per million indole-acetic acid

developed the red color when crushed in the HCl and iron perchloride reagent.

The auxin in question occurs but sparingly in soils, however, and is known to be formed by various fungi. The development of a pronounced red color in the hyphae when fresh sections of mycorrhizae were treated with the reagent was taken as evidence of its origin in the fungus. It was found that the addition of a few drops of amylic alcohol to the reagent would aid in the detection of traces of this growth promoting substance.

The color effect was most marked in the closely woven hyphal mantle, but it appeared in the vacuoles of hyphae intermeshed with the cortical cells as well as in the endodermis, pericycle, and some companion cells. That the auxin was produced in the fungus and translocated to the root was confirmed by the fact that crushed fresh materials from fungus-free leaders did not give the reaction, although it was noted that prolonged treatment was fol-

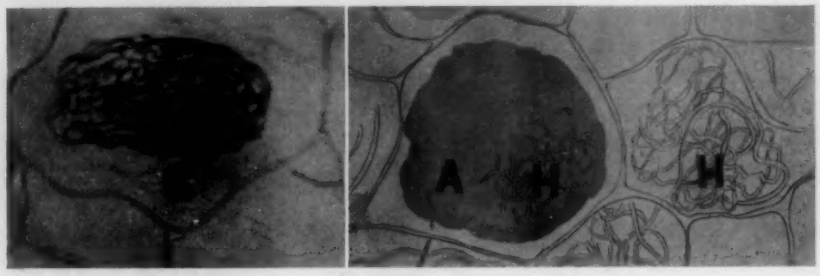


FIG. 5. Sketch of cells of cortex of Corallorhiza, with hyphae, H,H,H. Cell at left is from a fresh section treated with nascent indophenol blue. The central mass has become yellowish, while the peripheral portion has taken a pale blue, C, indicative of cytochrome oxidase, a reaction in actively growing cells. The contents of the fresh cell in the center with disintegrating hyphae, H, have become reddish after treatment with HCl and iron perchloride, A, by the presence of an auxin released by the hyphae.

lowed by the development of a reddish color, of a tinge different from the above, in the walls of wood cells.

As a supplemental test the reagent was applied to the tissues from the outer layers of "knees" of *Taxodium distichum*. Many cells of the cortex yielded a bright cherry red as well as the wood parenchyma, and the vacuoles of young cambium derivatives contained a reddish solution.

Sections from the thick mycorrhizae of Corallorhiza treated with the hydrochloric acid, perchloride of iron reagent, gave no perceptible color in the thin hyphae; but in cells of the cortex where the clumps of hyphae near the nuclei were disintegrating, the large vacuole contained a dense solution of reddish color tinged with purple, as if the auxin had been released with other soluble material by the fungus to the cell.

The actual amount of growth in the thick stem-mycorrhizae of the orchid is very small in comparison with the mycorrhizae of conifers and the supply of auxin found would be adequate. In what region this substance is metabolized has not been determined. Examination of the bud at the stage

when the scape begins rapid elongation would probably bring out more positive evidence.

Test for vitamin B_1

The blue color in vacuolar solutions following treatment of sections with the molybdic reagent is of a shade indicative of ionic or loosely held phosphorus as is the case in vitamin B_1, which includes pyrimidine and thiazol (fig. 5).

Test for vitamin B_6 (pyridoxin)

Fresh sections of coralloid roots immersed in a veronal (diethysodium-barbiturate) suspension of dichloro-quinone-chloro-imide results in the formation of pyridoxin-indophenol, in the vacuolar solutions, which comes down as a blue precipitate. The treatment should be carried out at temperatures not above 5° C. with the light excluded. (Phosphate or carbonate buffers at pH 7.6.) Pyridoxin will be found most abundant in the hyphae of the outer mantle in pine mycorrhizae (fig. 6).

Karyological features of roots

Cytological examinations of the tissues of roots of Pinus fixed in Helly's fluid confirmed the conclusion that the structure of the chromosomes, their behavior in leading roots under normal conditions, or after several years of isolated cultures, and in mycorrhizal roots was uniform. In the more actively growing tips, nuclei never reached the truly quiescent stage; after a telophase, and previous to the next prophase, nuclei retained a network of banded materials, with many chromocenters in the length of each chromosome. As the prophase occurs, the chromosomes lose their banded appearance, and the molybdenum reactions for nucleo-proteins at first obtained in the chromocenters, extend to include the whole chromosomes.

The resting nuclei of the differentiated tissues contain 4 nucleoli and 24 prochromosomes, corresponding to the 24 (the diploid 2n number) chromosomes seen in karyokinesis, as in the pines in general, 12 being the haploid number in the megosporangium (fig. 7).

It is to be added that neither the structure nor chemical reactions of the nuclei are demonstrably modified in pine mycorrhizae, in which the hyphal branches entering the cortical cells are palmated and are in close contact with masses of cytoplasm minutely vacuolated. Clumps of hyphae enclose the nuclei of cortical cells or the endophytic mycorrhizae of orchids; and the nuclei here are hypertrophied and densely granular (fig. 2).

Plastids

Two types of nucleo-protein-phospholipid inclusions occur in pine mycorrhizae—amyloplasts, and mitochondria—the last-named only occurring in the fungus. Both are capable of division and both give cytochemical reactions of phospholipids, are affected by acetic acid, alcohol, or lipid solvents, unless the lipids are previously oxidized to fatty acids by fixing reagents. As plastids become differentiated to take on the function of

synthesizing starch they become more resistant to solvents. These bodies are on the average bulkier in pine cells than the mitochondria.

In sections treated with the molybdic reagent both amyloplasts and mitochondria become blue (by the phosphorus reaction). Further staining with acid fuchsin results in the mitochondria becoming a bright red, while amyloplasts take on shades of purple of varying depth. Included starch grains become pure blue (figs. 4 and 7).

PHOSPHORUS METABOLISM IN MYCORRHIZAE

The assignment of an all-important rôle to phosphorus is justified by the fact that neither carbon nor nitrogen can be metabolized into cell-constituents without its action. In particular no proteins could be built up; the

FIG. 6. Sketch of partial cross section of coralloid root, vitally stained with 2–6-dichloro-quinone-chloro-imide. Hyphae, H, become a light blue as a reaction to pyridoxin (vitamin B_6) present. Hyphae traverse the cell walls but do not enter cavities of outer cells (I) rich in oxidized catechol compounds. Palmate branches are seen to enter cells of median cortex. Vacuoles of inner cortex take on various shades of red, indicative of catecholase activity, T, constituting a regional barrier to the fungus.

desoxy-pentose-nucleotides are essential constituents of nuclei, while the ribose nucleotides are equally important in the respiratory systems of living cells. Special attention was given to tests for compounds into which this element might be bound. The molybdic reagent which is especially useful for such tests was made up as follows: A. 20.9 ml. sulphuric acid was added to distilled water of the highest possible purity to make up 250 ml. 3 N solution in which 6.41 gm. of ammonium molybdate $[(NH_4)_6MO_7]$ was dissolved. B. 15.75 gm. sodium bisulphite was dissolved in 85 ml. of water, to which was added 0.5 gm. 1-amino-2 naphthol sulphonic acid ($C_{10}H_9O_4NS$) and

then 5 ml. 20 per cent. sodium sulphite solution which was stirred until complete solution was obtained.

Small amounts for treatment of fresh or paraffine imbedded sections were made by adding 1 ml. of B to 5 ml. of A. The best results were obtained by placing the preparations in a refrigerator at 5° C. for 24 to 48 hours. A progressively deepening blue color develops, appearing first at the site of ionic phosphorus in the vacuoles of the hyphae; later mitochondria become blue by the presence of this element in the phospholipidic boundary phase; nuclei throughout the section become a slaty blue at chromocenters made of nucleo-proteins. In general the density of the color was greatest at the site of ionic and loosely bound phosphorus. The deeper blue shade in the vacuoles was one which may be taken to indicate the presence of vitamin B_1.

Only through phosphorus linkages, achieved through phosphorus esterification, do most of the molecular structures coming into play in the cell metabolism acquire some degree of physiological activity or efficiency, as expressed in components of a respiratory system.

The hyphae in mycorrhizae are rich in proteids, polypeptides, or alpha amino acids, or more specifically in substances involving the radical COOH–CH–NH_2 as made evident from the purple color the mycelium develops when sections of mycorrhiza are heated in a solution of ninhydrin (triketohydrindene hydrate). The ectendotrophic mycorrhizal fungus is beautifully demonstrated as a purple network in between the cortical cells, which remain unstained; in the root only some phloem elements stain as a whole suggesting a translocation of soluble nitrogenous materials. From this histochemical picture it may be inferred that soluble forms of nitrogen from the mycorrhizal hyphae are mostly metabolized, presumably through carboxylation and phosphorylation, into the nucleoproteins which build up the tissues of the mycorrhizal roots.

As metabolism is so strictly dependent on phosphorylated compounds of the nucleotides or pentose nucleic acid-like components, it may be surmised that actively growing meristematic and postmeristematic tissues should show a high content in such compounds. In the course of tissue differentiation, as the affinity for basic dyes becomes less marked, so also the nucleic acid contents diminish.

Features of metabolism indicative of symbiosis

Hyphae coming from the investing felt around the short roots traverse the walls of the cortex with but little effect on the nuclei and cytoplasmic masses. Palmate branches penetrate some of the cells to make an arrangement facilitating nutritive exchanges, but the fungus does not penetrate the stele (fig. 6).

The stele in its earlier stage of differentiation from the meristem consists of elongated prophloem elements, the vacuolar solutions of which have a notably higher pH (staining orange with neutral red) than the surrounding tissues; the xylem with a conversely low pH, and the endodermis and peri-

cycle, have vacuolar solutions which become purple; they are characterized by a high polyphenolase activity, as is made conspicuous by the chromaffin reaction when sections are immersed in solutions containing potassium bichromate. The large vacuoles in both pericycle and endodermis are seen to contain catechol derivatives and catechol oxidases (figs. 6 and 8).

Absorption of salts by mycorrhizae

Mycorrhizal associations are formed by short lateral rootlets of conifers only, the leaders and secondary branches displaying the general features of

Fig. 7. Sketch of part of a longitudinal section of a main root of Pinus fixed in Helly fluid, imbedded in paraffin, stained with haematoxylin, and treated with I in KI. In one cell the nucleus, C, is at prophase surrounded with starch grains, S. Four nucleoli and 24 prochromosomes are present in others; amyloplasts enclosing starch grains and mitochondria appear in all cells.

a rapidly elongating apical region followed by a zone of root-hairs. As has been noted, young trees may survive for months or even years with such simple or normal absorption apparatus, without the formation of mycorrhizae. It is therefore possible to make valid comparative measurements of the rate or amount of absorption by mycorrhizal and non-mycorrhizal plants. In the most recent contribution to this subject Routien and Dawson (**21**) describe results in which mycorrhizal plants (*Pinus echinata*) were

found to show an increased absorption of K, Mg, Ca, Fe, and ionic phosphorus, which is to be connected with a heightened rate of oxygen intake—two to four times greater than in non-mycorrhizal plants, with correlated energy release and carbonic acid production.

Absorption of organic compounds by roots

The long cherished notion that only ionized material could be absorbed by roots has been gradually modified until it is now established that such complex substances as hormones, auxins, and vitamins may be taken from the soil by normal roots.

The present paper is concerned chiefly with absorption by mycorrhizal systems, in which hyphae act as the absorbing organs, capable of taking up nitrogen not only as NO_3 but as NH_3 or NH groups, linked into complex cyclic molecular structures. These humus derivatives are metabolized into nucleo-proteids, phospho-lipids and the various components of the cytoplasm and walls; surplus proteins may be accumulated in the hyphae. Uronic carbon in the soil may be in part residual or it may be synthesized by micro-organisms. Nothing is known as to its movement into hyphae, but it has been assumed that the polyuronides of the cell-walls of the cortex broken down by the action of the fungus are re-metabolized. Reliable evidence of the presence of any carbohydrate except glycogen in hyphal cells is lacking.

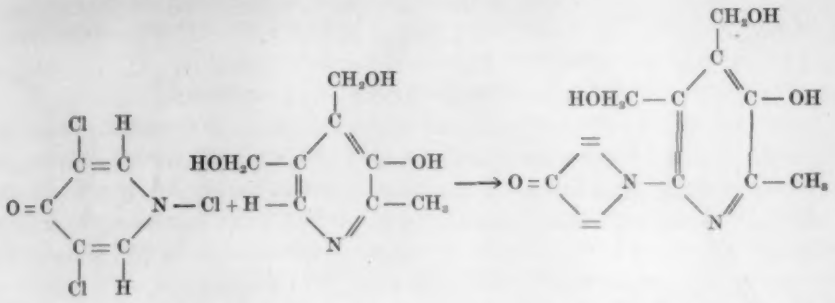
Growth-factors and carbon metabolism

All the process of survival and growth in the excised root tips must depend ultimately on the catalysts, which govern the process of biological oxido-reductions, whereby energy is obtained, enabling the plant to carry on the process of living.

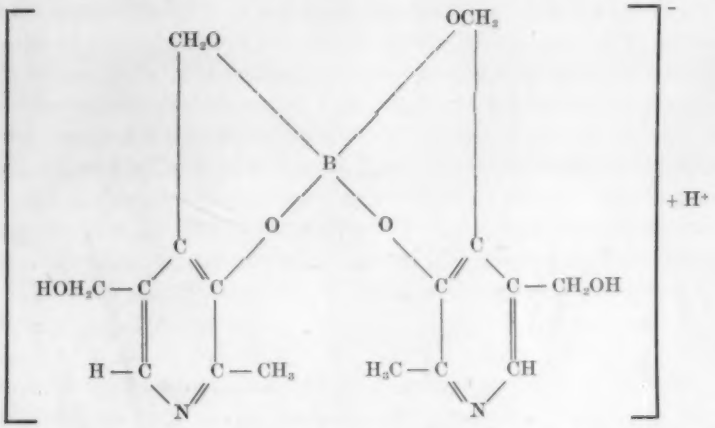
Two types of such systems may be distinguished: (1) The protein-alloxazin system, where the alloxazin derivative is so firmly bound to the protein that it can be set loose only by dilute acid hydrolysis. To this type belongs one of the components of the vitamin B_2 complex: "riboflavin." (2) The protein-pyridine nucleotide systems, where the pyridine nucleotide represents a "prosthetic group" easily removed by washing, and which has long been described as the "co-enzyme" or "coferment."

The best known coferments which assist in the transference of hydrogen, are the so-called "hydrogen carrying coferments," which are a tri- or bi-phospho-pyridine-nucleotide. The former contains 3, and the second 2 mol. phosphoric acid, besides which, each of these two nucleotides consists of a molecule of the amide of nicotinic acid, 1 molecule of adenine, and 2 molecules of pentose. To survive and grow, isolated roots must synthesize, or obtain from their mycorrhizal partners, or from the soil, these pyridine nucleotides. Pyridine has been assumed to be a significant source of nitrogen for soil organisms, thriving in humus; it may yield nicotinic acid by the introduction of a carboxylic group at carbon 3 in the cyclic chain. It is to be noted that two of the vitamins, nicotinic acid and pyridoxin (vitamin B_6) are pyridine derivatives.

2–6-dichloro-quinone-chloro-imide condenses with pyridoxin in the presence of appropriate buffers to yield indophenol blue.



This reaction does not occur in the presence of a borate ion, while building a complex involving the 3-hydroxy and 4-hydroxy methyl groups.



Pyrimidine, one of the two parts of the molecule of vitamin B_1, has recently been found to protect carboxylase against the enzymatic action of the phosphatase which tends to destroy it.

Pyrimidine, as associated with thiazol in the molecule of vitamin B_1 or thiamin, plays a dual rôle—that of a growth substance and that of a control of cell fermentation.

Elaboration of carbohydrates

As to vitamin B_1 or thiamin, made up of the two constituents pyrimidine and thiazol, it plays a most essential rôle in the metabolism of carbohydrates, since, when phosphorylated into the diphosphate of thiamin, it becomes the co-carboxylase.

Vitamin B_1 has been chiefly considered in connection with the decarboxylation process; Evans called attention to the fact that vitamin B_1 also controls the reversible reaction of carboxylation, being apparently directly concerned with the C–C linkage whereby pyruvic acid can unite with carbon

dioxide to yield oxalacetic acid: enzymatic fixation of carbon dioxide in oxalacetate has been recently confirmed by KRAMPITZ *et al.* (**13**).

Pyruvic acid can unite with carbonic acid to yield oxalacetic acid $COOH \cdot CO \cdot CH_3 + CO_2 \rightarrow COOH \cdot CO \cdot CH_2 \cdot COOH$. This carboxylation of pyruvic acid may then be followed by the following:

4 oxalacetic acid → alpha ketoglutaric acid + 2 malic acid + 3 CO_2.

The widespread occurrence of the carbon dioxide effect may be taken to indicate that carbon dioxide takes part in a fundamental metabolic process, common to many organisms. The synthesis of oxalacetate is a reaction sufficiently fundamental to explain the use of carbon dioxide, although, experimentally, it should be possible to replace carbon dioxide by malate, by fumarate, or asparagine or other substance which can serve as a source of oxalacetate.

Carbon dioxide is an active metabolite in heterotrophic cells; it can condense with pyruvic acid to form oxalacetic acid. By this reaction carbon dioxide is "assimilated." Oxalacetic acid has at least two important functions in cell life: it acts as a hydrogen carrier in energy-giving processes, and is a parent substance to a number of cell constituents.

Through carboxylation, pyruvic acid acquires two additional carboxyl groups. These lend to the two middle C atoms the high reactivity which is necessary for the catalytic function. Both carboxylic groups were demonstrated by SZENT GIORGYI to account for the absorption of the C_4 dicarboxylic acid on protoplasmic surfaces. Their high absorbing capacity of the traces of C_4 dicarboxylic acids present, saturates the enzyme and maintains respiration, a function of especial importance in the isolated root cultures.

STARCH SYNTHESIS

Synthesis of starch from phosphorylated soluble carbohydrates with the release of phosphorus in simple form as set forth in the reversible equation of HANES, takes place in the amyloplasts with a consequent reduction of the osmotic pressure of the cell liquids.

In the reversible equation, defined by C. S. HANES: "Starch + inorganic phosphate ⇄ glucose-1-phosphate," the equilibrium is determined by the divalent ions,

$$(HPO_4)^{=}/(C_6H_{11}O_5 \cdot OPO_3)^{=}$$

The metabolic turn-over of phosphate may therefore involve a series of recurrent phases, which might be as follows: (1) absorption of inorganic phosphate from the soil; (2) introduction of inorganic phosphate into ester linkages within the mycorrhizal fungus; (3) generation of energy-rich phosphate bonds by oxidation-reduction; (4) taking over and distribution of such bonds by the catalysts in the fungus tissue; (5) utilization of these energy-rich phosphate bonds, and concomitant liberation of inorganic phosphorus; (6) translocation of such inorganic phosphorus to the pine tissue, where the above phases, 2 to 5, would again occur.

In other words, the mycorrhizal association would be one wherein the

fungus partner would obtain inorganic phosphate (as well as nitrogen and carbon) from the soil, and be able to build high energy phosphorus linkages, thereby maintaining a high potential level; it would then release inorganic phosphate which would eventually be carried into the irreversible group acceptors of the pine tissue.

In accordance with widely accepted views phosphate current can be used to do osmotic work, to synthesize cytoplasmic materials, nucleic acid, and to carry on organic chemical synthesis in cells; all such reactions involve group donators and group acceptors. The force which drives the group into the final acceptor may be the difference in potential levels.

Histochemical map of mycorrhizae

Determination of the sites of many important metabolic transformations as described in previous sections has yielded some identifications of regions of the principal activities of the symbionts. In some cases the appearance of untreated sections furnish reliable indications of the nature and action of substances in a tissue. Thus the brown discoloration of the vacuolar contents of outer cortical cells engaged with mycorrhizal mantle is to be attributed to the irreversible oxidation of polyphenols, mainly pyrocatechol, to the corresponding quinone.

Under normal active cells, pyrocatechol is protected by reducing substances; but if these substances become oxidized, the catechol is also oxidized and undergoes molecular aggregation of the gummy, brownish catechol tannins which stain red in neutral red in living cells and retain the color after death.

Vacuoles in one and the same cell may differ in the composition of the solutions or colloidal dispersions which they contain. Some positive reactions indicative of their character were obtained by vital staining. It is important that the reagent be isotonic and isoionic to the vacuolar solutions in this treatment. To this end neutral red was added to a Ringer's solution made up by adding 6 gm. sodium chloride, 0.075 gm. potassium chloride, 0.1 gm. calcium chloride, and 0.1 gm. sodium bicarbonate to 1 liter of distilled water. Cells of mycorrhizae remained alive in this reagent at ordinary room temperatures during the period of observation with accumulation of the dye in the vacuoles, the nucleus and cytoplasm being unstained.

The development of the capacity of the vacuolar fluid to absorb neutral red is taken to be concomitant with the formation of polyphenols; a similar intensification of reaction occurring with either 2–6-dichloro-quinone-chloroimide, potassium bichromate, or the molybdenum reagent.

The progressive effect of increasing polyphenols was observed in sections of the apical parts of roots. No vital stain was absorbed by the meristematic cells, but as differentiation proceeded, vacuoles enlarged and coalesced and their contents became reddish, the elongated cells about the xylem becoming deep purple and the phloem a light orange red, indicative of polyphenols and also of the higher pH in the phloem, this difference also being applicable to the color in the hyphae (fig. 8).

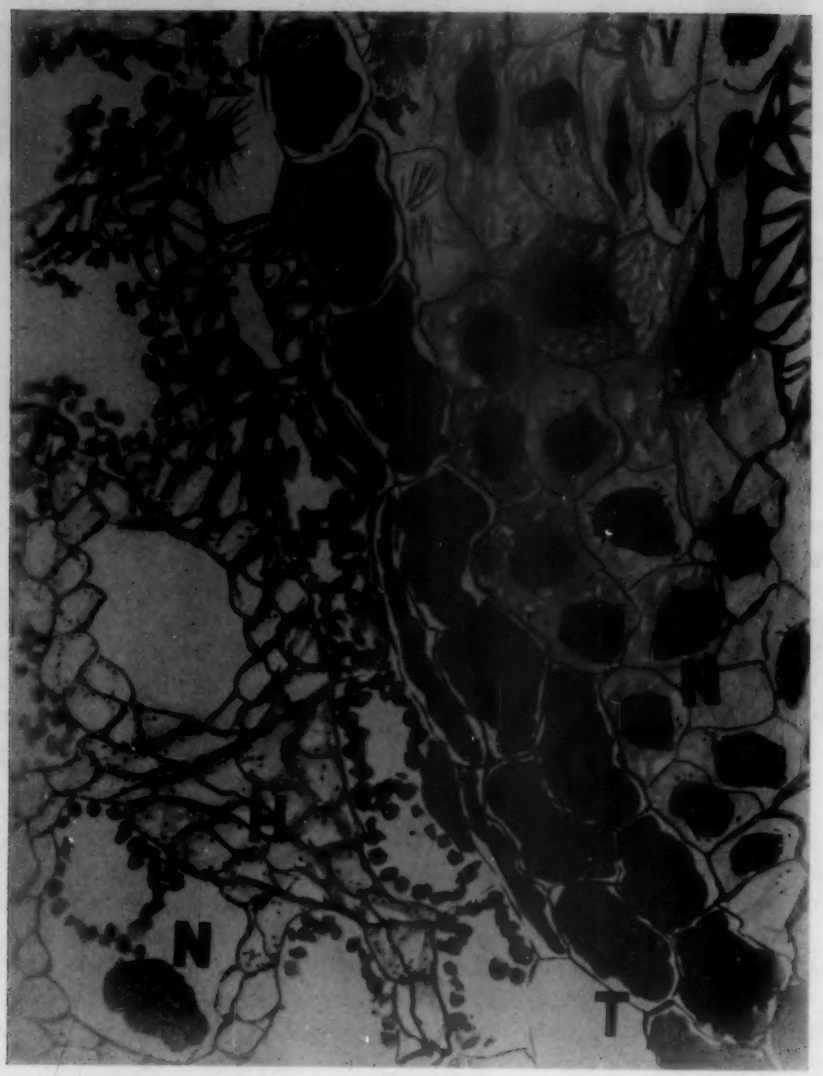


FIG. 8. Sketch of section in apical region of coralloid root of pine in isolated soil culture, collected June 5th, fixed in Helly fluid and stained with acid fuchsin and methyl green. Hyphae, H, at left have finely vacuolated cytoplasm staining pink, with numerous mitochondria becoming red. The endodermis contains yellow phenolic precipitates, P, and the vacuolar contents of the pericycle are rich in catechol tannin, T, which stains deeply. Crystals of calcium oxalate are seen in some of the older (upper) cells. Parenchymatous cells of the stele to the right are in a meristematic condition with large nuclei, N, and many small vacuoles, V. Terminal portions of wood cells are at the upper, right margin.

COACERVATES

In addition to the series of colloidal interphases of the first order as implied in the preceding paragraphs, interphases of a second order come

into existence when two phases separate from within a vacuolar solution. When such two phases become permanently separated by a membrane so that each phase and the membrane or interphase differ in physico-chemical properties, especially in oxidation-reduction potentials, bodies or masses are formed which are now termed *coacervates.*

Further examination revealed that the membrane was phospho-lipidic and alveolated by the retention of some of the water in which its material was dispersed; the brown central core was composed of oxidized phenols. The nature of these bodies, known as coacervates, was established by DE JONG (**12**) and has been discussed by DUFRENOY and REED (**5**).

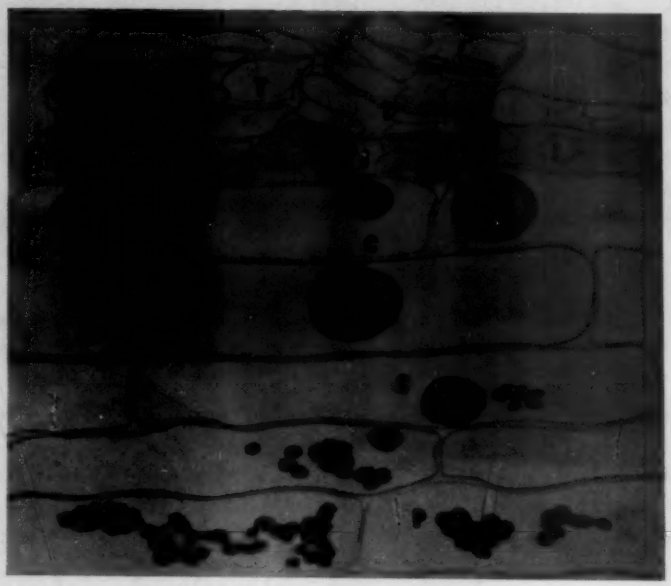


FIG. 9. Sketch of part of a longitudinal section of the apical region of the main root of pine stained vitally with neutral red. Initial cells as well as young derivatives, T,T, did not take the stain. The vacuolar contents of older cells, V,V, became reddish, with the uncolored nuclei, I,I, compressed into star-shaped bodies. In cells of more advanced stage large spherical inclusions, C,C,C, showed a differential staining of the boundary phase and the core characteristic of coacervates and indicative of colloidal structure. Phenolic material in solution in other cells came down as precipitates.

When cells of the root-cap of pine were vitally stained with neutral red, the presence of small globular bodies was revealed. The limiting membranes of these bodies became a deep red, while the enclosed central mass was of a lighter red. Post-vital staining with 2–6-dichloro-quinone-chloro-imide brought into view large spherical refringent bodies, the boundary layers of which were dark brown while the central part was a lighter hue, which may be interpreted as indicative of a strong polyphenolase activity.

The vacuolar colloids have been generally considered as electronegative, but some of them (phenolic compounds or their derivatives) may be assumed

to be amphoteric. Electro-positive charges may be supposed to develop within the vacuolar colloids in connection with oxidation-reduction phenomena. Where an oxidase system exists in high concentration, there might be a locus for the attraction of cations; and conversely, where the dispersal of cations is concomitant with oxidation or dehydrogenation, phenolic compounds form molecular linkages, resulting in the formation of larger and larger molecular aggregates. If these aggregates remain firmly bound to the suspending saline liquid or serum, there may result a process of formation of definite physical bodies such as that described by BUNGENBERG DE JONG as due to coacervation. These condensation products show the microchemical characteristics of polyphenols; more specifically, inasmuch as they form pyridoxin-indophenol complex with 2–6-dichloro-quinone-chloro-imide in phosphate or veronal buffers, they may be considered to be composed largely of pyridoxin. They exhibit the following characteristic physicochemical activities (fig. 9):

1. A high level of oxidase, as evidenced by the rapid oxidation of polyphenols or amides to their colored derivatives, and concomitant high reducing power, as evidenced by the reduction of the silver ion from silver nitrate to metallic silver, or of mercuric chloride, to mercury, or of osmic acid to osmium.

2. Attraction of cations, explaining staining with basic dyes.

3. Dispersion of anions, explaining why phosphate anions move out into the suspending liquid.

4. The formation of the precipitation membrane at the periphery of the condensed colloids, explaining why phosphatids or phospho-lipoids, being amphoteric, remain at the periphery.

Under what may be considered as the "normal condition" the vacuolar solution appears to be a random distribution of ions and of molecules, where an rH lower than 12 is maintained, so that phenolic compounds such as catechol are protected from permanent oxidation to quinone derivatives and polymers. The vacuolar solution contains both the oxidizable polyphenols and the system which can catalyze their oxidation, in other words, the polyphenol oxidase. As previously mentioned, these polyphenols are normally protected from oxidation by the presence of the ascorbic-dehydroascorbic, or the dihydroxy-maleic acid systems, and chiefly by the presence of such thiol containing groups as cystein and glutathione, the latter of which is able to act as a "reservoir of hydrogen." Under conditions as yet undefined, the random distribution of substances no longer prevails, and cations become concentrated about some focus from which anions are repelled. The site of cation concentration is also the site for a high level of oxidase activity and can be demonstrated in the case of catechol oxidase or polyphenol oxidase, which is a copper protein whose activity is a function of the concentration of Cu^{++}. Therefore we must expect to find a strong positive response for the ortho-diphenols such as catechol and their derivatives and for phenols such as pyridoxin; a strong reaction for polyphenol oxidase and a correlative

strong reaction for Cu may be expected in the vacuolar inclusions. Also a negative reaction for anions such as PO_4, while a positive reaction for bound phosphorus could be expected in amphoteric phospholipids at the interphase between the vacuolar inclusion and surrounding vacuolar solution. As a result of the high activity of polyphenol oxidase and dispersion of the dehydrogenase system (involving probably nucleo-proteins with – SH active groups) oxidation and concomitant polymerization of the orthodiphenols to gummy derivatives composed of glucosides of the anthocyanol type would be possible as may be observed in epidermal cells of some flowers. These molecular linkages resulting from carbon bonds, through dehydrogenation should be reversibly broken by hydrogen donors which would therefore appear to be solvents of the inclusions, *e.g.*, alcohols, and, in general, liquids with methyl or ethyl groups. The previous "fixation" of plant material with oxidizing solutions which contain potassium bichromate protect these inclusions against solvents.

Discussion

A study of ectendotrophic mycorrhiza under natural condition and in experimental cultures yields ample evidence that the fungus is not parasitic. Isolated segments of the root system, with mycorrhizal branches, a few cm. in extent, have been seen to carry on an existence for many seasons similar to that of a chlorophylless plant. The older parts of the main axes die away, the leaders extend, new mycorrhizae are formed and surplus starch is continuously accumulated. A small fraction of the salts and organic material derived from the soil may be absorbed by the zone of fragile root-hairs following the extension of the leaders, but the greater part of the material is taken up by the hyphae and, after some metabolization, is translocated into the cortical cells of coralloid roots.

Auxin (indole-acetic acid) is formed in abundance in the hyphae; vitamin B_1 and vitamin B_6 may be traced from the origin to their presence or effects in the root.

The implied processes may be keyed to the metabolism of phosphorus. This element absorbed from the soil in inorganic form enters into water-insoluble combinations, such as the nucleo-proteins from which it may be freed by phosphatases; acting concomitantly with dehydrogenases from the hyphae it passes into the root-cells, making similar combinations from which it is freed by respiration to migrate to the stele, where it is again bound in stable unions in a region of low pH and low oxidase activity.

In the over-all nutritive cycle, mycorrhizae add to the material entering the roots and passing upward to furnish energy and structural material to the tree; leaves, fruits, bark, twigs, etc., are cast off to contribute to the general layer of humus which is the habitat of the fungus. A minute fraction of the mycelia becomes engaged with the roots to which simple and metabolized soil substances are transmitted.

Hyphal branches extending in the middle layers of cortical cell walls, have been assumed to metabolize for their own nutrition plastic materials,

or more precisely, the polyuronides from the cell walls. In addition, such mycorrhizae as that of the pine send other modified branches into the cell which become enmeshed in a reticulum of cytoplasm amidst a cluster of minute vacuoles. This arrangement with the chemical reactions described indicate a region of active translocation of amino acids.

An analysis of the metabolic processes of mycorrhizae makes it evident that practically all of the material which enters into the construction of cells of isolated roots or of chlorophylless plants is translocated from the fungus. Metabolization of this material in the higher plant, in addition to nitrogenous material, produces cellulose, sugars, and starch, not found in the fungus, in the significant presence of plasts. Glycogen, known to occur in hyphae, but not identified in the observations described here, is non-diffusible but, if present, its products by the action of amylolytic ferments might be translocated to be synthesized as starch. The protoplasmic organs of hyphae include the main components of cells of higher plants except carbohydrates and plasts. Auxins, and vitamins of the B group are seen to accumulate in, or to be originated by, the fungus and to be transmitted to other parts of the mycorrhiza.

Lastly, it is to be said that the presence or action of the fungus is not necessary for the germination of pine seeds. The seedling may not form a mycorrhizal alliance with the fungus until the second season, but the implied symbiosis is indispensable for the normal maturity of the tree.

The non-necessity of the fungus for germination of terrestrial orchids has been wrongly taken as a proof of parasitism of the fungus by J. F. Curtis (**3**).

A review of previous work by the senior author and the results of recent examinations of the mycorrhiza of an orchid, *Corallorhiza maculata,* supports the view that the fungus (Rhizoctonia) which enters most numerously into nutritive associations with species of this family sustains a symbiotic relation similar to that of the elements in the pine mycorrhizae, although the morphological engagements of the fungus and seedplant are widely different. The mycorrhiza of orchids is of the endophytic type which in some cases is accompanied by striking modifications of the roots and shoots.

Parasitic fungi may infect the roots and stems of some orchids, but when the association follows a regularized pattern accompanied by a loss of absorbing organs and photosynthetic apparatus, the assumption that the fungus yields nutritive material which is usually furnished by these organs is acquired by symbiosis with the fungus, is not only allowable but inevitable.

Adult individuals of Corallorhiza have no roots; (whether these organs are formed in germination is not known) and its leaves, represented by bracts, contain so little chlorophyll as to be functionally negligible.

The amyloplasts, however, are seen to function normally as evidenced by the dense accumulation of starch in the coralloid stems.

The fungus partner gains entrance to the cortex of the stem at some early stage, and the hyphae follow the development of this tissue in the

early post-meristematic stage. Once infected the partnership is for life; unlike the pine mycorrhiza, as the fleshy stems die at the base after the fashion of a rhizome, and extend at the apices of the branches, the extending hyphae send branches outwardly which emerge from the epidermal cells, constituting the absorbing mechanism of the system. The principal processes of metabolism of pine mycorrhiza, including a dense accumulation of starch, are duplicated. In this case, however, the hyphae form dense swirls against the nucleus, which shows a modified granular structure; interchange between this organ and the hyphae appears to take place instead of by a vacuolar mechanism as in the pine. Positive reactions for auxin are shown only in the older cells, in which the fungus appears to disintegrate and yield this and other contents to the cortical cells.

Summary

1. Results of a comparative study of the endophytic mycorrhizae of terrestrial orchids and of the ectendotrophic mycorrhizae of pines are presented.

2. The possible mutative origin and vegetative propagation of "stem-mycorrhizae" as exemplified by Aplectrum is described.

3. Association of pines with symbiotic fungi can not be connected with any heritable morphological alterations. Short lateral rootlets are invested directly by hyphal branches of mycelia in the soil; such association may not be made until the second season.

4. No plants of Corallorhiza with roots or other than stem-mycorrhizae have been seen. Results of observations of germination, development of seedlings, and beginning of symbiotic association with fungi are not available.

5. The mycelium of the endophytic mycorrhiza of Corallorhiza is in the cortex, to which it gains access in an unknown early stage; in its growth it keeps pace with the differentiating meristem, sending branches outwardly through the epidermal cells of underground coralloid branches in simulation of the arrangement of root-hairs. Symbiotic fungi of other terrestrial orchids with roots traverse these organs in a similar manner, sending hyphae out into the soil through the durable root-hairs and other epidermal cells.

6. The absorption of inorganic phosphorus from the soil by the fungus, and the stages in its metabolism terminating in the stele of the root, identifiable origination in hyphae and translocation of auxin, vitamins, and amino compounds to the root-tissues, together with the capacity of isolated segments of mycorrhizal roots to survive and grow like a chlorophylless plant, establishes the non-parasitic character of the fungus.

7. The distribution of the hyphae in the cortex of pines does not extend beyond the endodermis in which catechol tannins are formed; the middle lamellae of the outer cortex is traversed and its disintegration products are available for absorption.

8. Palmated branching hyphae enter cortical cells and become closely engaged with a cytoplasmic mass characterized by minute vacuoles sugges-

tive of a mechanism of exchange. Hydrolyzation products of polyuronides, of starch, and of other diffusible compounds may be absorbed by the fungus.

9. The mycelium of the endophytic mycorrhiza advancing toward the growing apex of coralloid branches forms dense swirls of hyphae enveloping the nucleus which is hypertrophied and densely granular, constituting an arrangement for possible exchanges. The masses of hyphae disintegrate in the older parts of the stem, their contents becoming available to still living cortical cells as exemplified by reactions for auxin. Layers and islands of cells containing oxidized catechol form a barrier to the extension of the hyphae.

10. The hyphae of the fungus partner and the cells of the symbiotic seed plant are richly endowed with mitochondria appearing as short rods or cylindrical bodies, giving reactions for phospholipids. No cytochemical results yield any clue as to their rôle in the activities of the cell. The cells of the higher plant also include plasts which may be differentiated to synthesize proteins or starch; these capacities are exhibited by the plasts of isolated pine roots in soil cultures.

11. Carbohydrate metabolism takes place mainly in cortical cells and in the presence of plasts. Auxins and vitamins are accumulated by, or originate in, hyphae and are translocated to the roots.

12. Pads of material at the pores of cross walls of hyphae demonstrated by diverse reagents and stains were observed. Possible similarity of composition and function with sieve plates of higher plants is noted.

13. The limited initial growth of the short laterals of pine roots which become invested by the mycelium of the symbiotic fungus, may be connected with the lack of cytochrome oxidase which is active in the meristem of rapidly elongating leaders. The subsequent profuse coralloid branching of the short roots is in the presence of such oxidases produced by the hyphae.

14. The phenolic compounds in the pericycle and endodermis which are firmly held in suspension or solution in the vacuolar solutions or coacervated into spherical masses with a phospholipidic membrane in the endodermis and pericycle, are neutral to the protoplasm in these elements. When allowed to escape from the vacuoles the phenolic substances oxidize to tannin which "fixes" the protoplasm as seen in the brown peripheral layers of old mycorrhizae.

CARMEL-BY-THE-SEA
CALIFORNIA

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CALCIUM AND OTHER POLYVALENT CATIONS AS ACCELERATORS OF ION ACCUMULATION BY EXCISED BARLEY ROOTS

FRANK G. VIETS, JR.

(WITH ELEVEN FIGURES)

The effects of one ion on the absorption of another has merited the attention of physiologists for almost a century. The investigations in this field (**15**), however, have usually been concerned with the analysis of plants grown in different media or with plants or cells subjected for short periods generally to high concentrations of salts with little regard for the metabolism of the tissue. Growth experiments are so complicated by secondary factors that they are of little value in the study of the primary phases of absorption. Short term experiments with inactive tissues and high concentrations of salt (passive absorption) have led to the theory of antagonism between monovalent and divalent cations.

The technique of growing barley roots (**6**) capable of rapid absorption and accumulation of mobile ions against concentration gradients during intensive aerobic metabolism offers a new and valuable method for the study of ion relationships. The object of this paper is to present data on the absorption of K and Br by barley roots as influenced by various polyvalent cations and to discuss them in relation to current theories of solute absorption. A brief account has been published by the author (**17**).

Material and methods

The method of growing root material and general technique of experimentation developed by HOAGLAND and BROYER (**6**) have been followed in these investigations. Sacramento barley was grown for about 4 weeks in a complete culture solution with a limited supply of nutrients. The roots were then excised, subjected to various absorption treatments, frozen, and later thawed and the sap expressed for analysis. These roots, low in total salt, high in sugars and capable of rapid accumulation of K and Br were in no sense deficient in Ca. Hence, these experiments cannot be compared with those in which Ca supply was deliberately restricted in order to study Ca as a permeability factor.

The plants were grown in the fall, winter, and spring months and hence the root weight, salt content, and capacity for salt accumulation fluctuated as indicated by HOAGLAND (**5**), but it is believed that this variable does not invalidate any of the conclusions. Modifications in technique necessary for certain experiments will be noted later. In all cases where very dilute (0.0001 N) KBr solutions were used the roots were immersed in tanks holding at least 40 liters of solution in order to provide enough total salt so that the concentration would not appreciably diminish during the course of the

absorption. The pH of the experimental solutions varied within narrow limits (5.5 to 7.0), a range exerting little effect on ion accumulation (7).

Results

General features of the calcium effect

Several experiments have shown that the classical concept of antagonism of Ca and K in absorption does not apply to barley roots accumulating salt from dilute solutions. The results of a typical experiment are shown in figure 1. Sets of roots were immersed in a series of 0.005 N KBr solutions containing $CaBr_2$ varying from 0.0001 N to 0.5 N with 0.005 N KBr serving as a control. After 8¼ hours the roots immersed in 0.3 N and 0.5 N $CaBr_2$ had lost 10 and 20 per cent. of their original fresh weights, respectively. The lower absorption and the loss in weight was probably due to unfavorable

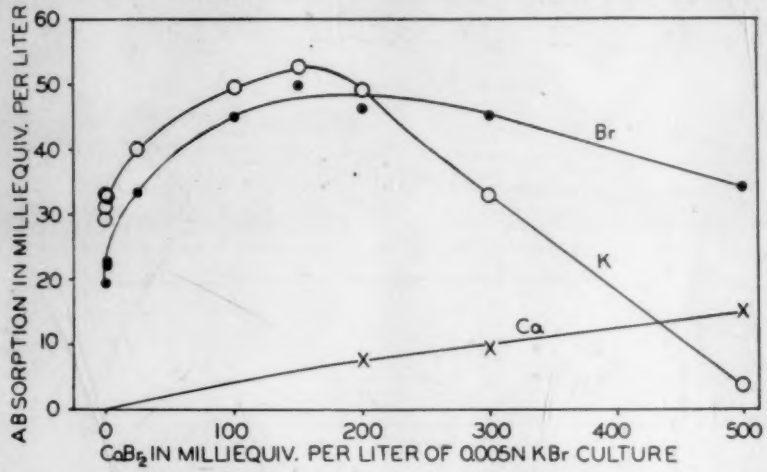

Fig. 1. Absorption of K, Br, and Ca in the expressed sap of excised barley roots placed in aerated solutions as indicated for 8¼ hours.

osmotic effects. Roots from the other solutions were normal in appearance and showed no significant change in weight. The significant results of this experiment are that $CaBr_2$ increased the amount of K absorbed by about 100 per cent. and the Br absorption by more than 150 per cent., the increase depending on the $CaBr_2$ concentration. The absorption of Ca was small and the Ca concentration in the expressed sap was always lower than that of the external medium except for the 4 most dilute solutions. Several experiments of this kind have shown the same favorable effect of Ca on absorption, whether the data be evaluated on the basis of the expressed sap or on the total root ash. The concentration of the Ca, K, and Br remaining in the press cakes was of the same general magnitude as that of the expressed saps [Compare Broyer and Hoagland (3)].

This experiment is of interest in view of the contentions of Mazia (14), working with Elodea leaf cells, that Ca had a higher capacity to compete for

positions on the protoplasmic membrane than K and thus antagonized the entrance of K. The above experiment shows that a Ca : K molecular ratio of 15 : 1 produced the largest absorption of K and a ratio of 30 : 1 gave as large absorption as KBr alone.

Another experiment using a range of $Ca(NO_3)_2$ concentrations up to 0.1 N in a standard solution of 0.005 N KNO_3 has shown that Ca associated with a different anion than Br is also able to increase the absorption of K by about 80 per cent. A similar effect was obtained with solutions of $CaSO_4$ up to 0.025 N in a solution of 0.005 N K_2SO_4. The increase in the K absorption without a corresponding absorption of anion was compensated in the expressed sap by an increase in the total organic acid anions as previously shown by ULRICH (**16**) for the differential absorption of cation and anion. The total organic acids were calculated from the titration curves of the expressed sap according to the method of ISAACS and BROYER (**10**).

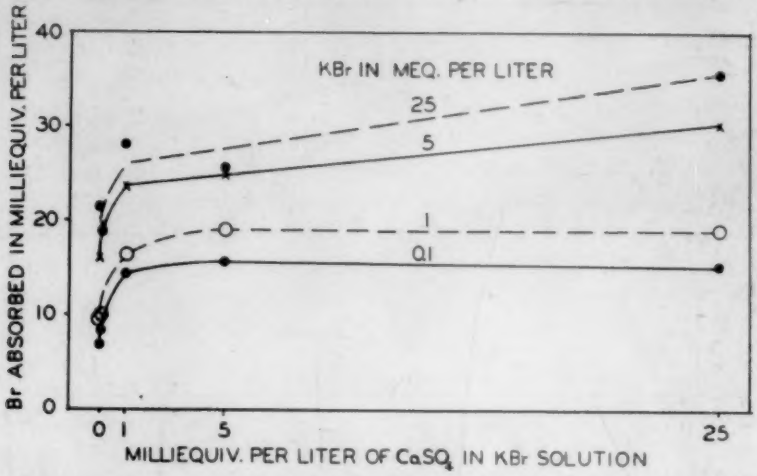

FIG. 2. Bromide absorbed in the expressed sap of barley roots immersed for 10 hours in KBr solutions at different $CaSO_4$ concentrations.

In the foregoing experiments 0.005 N K solutions were used in conjunction with variable concentrations of Ca and the solution contained only one species of anion. It was conceivable that the high and variable concentration of anion might influence in some way the absorption from the constant amount of K used. Hence, the experiment shown in figure 2 was performed in which different concentrations of $CaSO_4$ were used in conjunction with various levels of KBr. The data for Br absorption show that the Ca ion is significant in the enhanced absorption rather than a high common anion concentration (fig. 1). Also apparent is the fact that 0.001 N $CaSO_4$ gave marked increases in absorption of Br at all KBr concentrations. It thus appears that the concentration of Ca is of importance rather than any particular ratio of Ca to K and Br.

These experiments indicate that the traditional concept of antagonism of Ca for monovalent ion absorption from dilute solutions cannot be applied

to accumulating barley roots of the type used in these experiments. They may further indicate that if the first step in the accumulation of ions is an adsorption on certain adsorption positions of the membrane as postulated by many investigators (**2, 4, 13, 14**) then either Ca is unable to compete for these positions or else it increases in some unknown way the total number of positions thereby obscuring the competitive effects. The view that Ca is unable to compete with K for adsorption positions is consistent with investigations on ion exchange between roots and media. JENNY and OVERSTREET (**11**) found that Ca bentonite or Ca salts of equivalent concentration of Ca did not remove as much K from barley roots by exchange as did the corresponding NH_4, K, and Na sols or solutions. Similarly, BROYER and OVERSTREET (**4**) found that radioactive K in roots would not exchange for Ca in $CaSO_4$ solution whereas it would exchange readily for non-radioactive K in the solution.

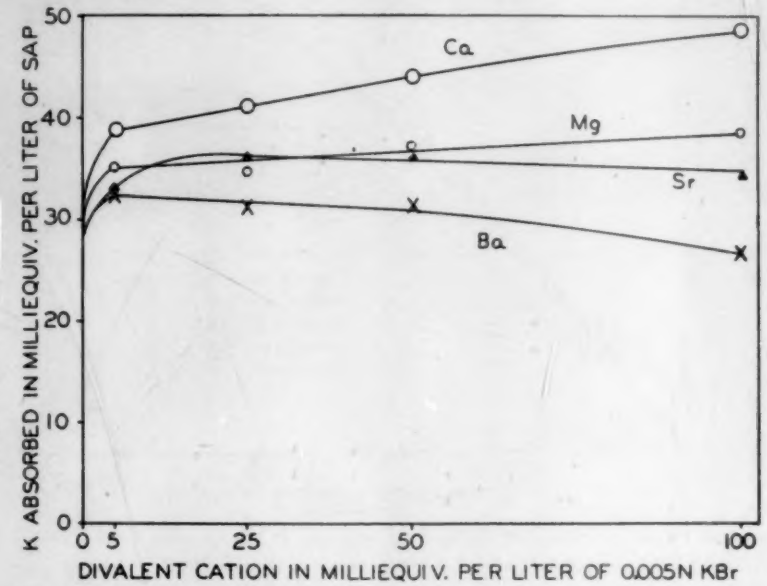

FIG. 3. Influence of various divalent cations on the absorption of K in the expressed sap of roots kept in the solutions indicated for 8¼ hours.

EFFECTS OF OTHER POLYVALENT CATIONS

Since Ca promoted absorption of K and Br it was of interest to learn if other ions like Mg, Sr, Ba, and possibly Al might also be effective and if the relative effect of these ions could be arranged in a series corresponding to their known physico-chemical properties.

A preliminary experiment using 0.005 N solutions of $Ca(NO_3)_2$, $Mg(NO_3)_2$, $Sr(NO_3)_2$, and $Ba(NO_3)_2$, respectively, containing 0.005 N KNO_3 showed that these divalent cations could also accelerate K accumulation, the magnitude of the increase being 7 to 11 m.eq. per liter of additional K absorbed in the expressed sap of the roots.

A second series using $MgBr_2$, $CaBr_2$, $SrBr_2$, and $BaBr_2$, respectively, in a range of concentrations from 0.005 N to 0.1 N in a solution 0.005 N with respect to KBr gave marked increases in the amount of Br absorbed; but only Ca, Mg, and Sr increased the K absorbed over that of the controls as shown in figure 3. The addition of $BaBr_2$ had little effect on the amount of K absorbed. There were no visible signs of root injury and the roots showed no significant changes in weight.

One of the foregoing experiments showed that the absorption of Ca is quite small in relation to the K and Br absorbed concomitantly; but it was possible that Sr, Ba, and Al, which are toxic to the growth of plants, could

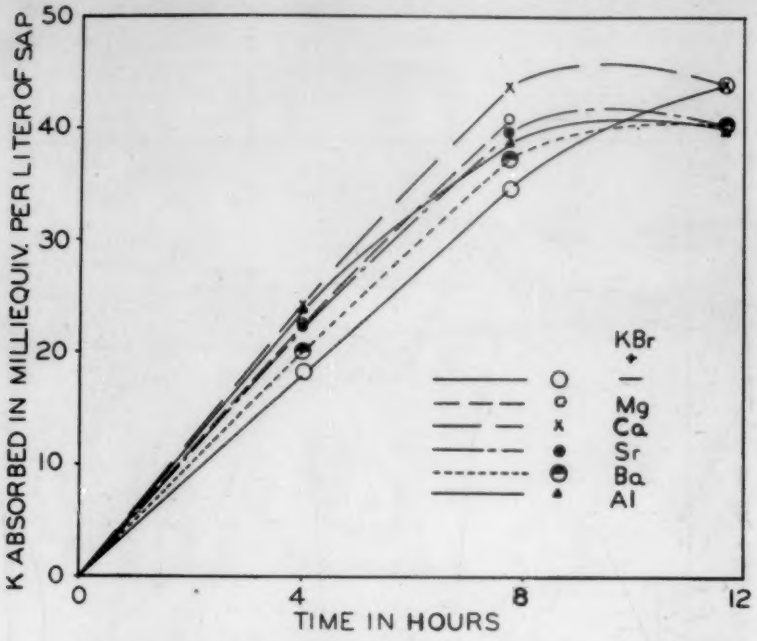

FIG. 4. Potassium absorbed in the expressed sap of barley roots from 0.005 N KBr as modified by dilute concentrations of polyvalent cations.

be absorbed in sufficient quantities so that their deleterious effect might mask their favorable effect on the plasma membrane. Therefore, an experiment using short periods of time and low concentrations (0.001 N) of Sr, Ba, and Al ions in order to minimize their absorption was conducted. The data for K and Br absorption are shown in figures 4 and 5, respectively. These curves show that even in low concentrations these ions are effective, but that the differences between ions are of insufficient magnitude for any arrangement in a series. Another experiment using 0.001 N $Al_2(SO_4)_3$ in a solution of 0.050 N KBr has shown that K absorption is increased by 10 and Br by 20 per cent. over the controls.

These experiments, in general, show that Ca of the cations tested is the most efficient accelerator of accumulation, but that others can also perform

the same function. Any attempt to arrange them in a series must involve careful consideration of the many variables and utilization of new and improved techniques.

It was of interest to know if these cations were performing independent or some common functions. A series of solutions were made in which the $CaSO_4$ concentrations varied from 0 to 0.025 N in a solution of 0.005 N KBr and the $MgSO_4$ concentrations varied in the same series from 0.025 N to 0 in an inverse order. Thus the solution always contained 0.005 N KBr and a total concentration of $CaSO_4$ and $MgSO_4$ amounting to 0.025 N with a varying ratio of Ca to Mg. The results for K and Br absorbed from the solutions as shown by the expressed sap are plotted in figure 6. This experi-

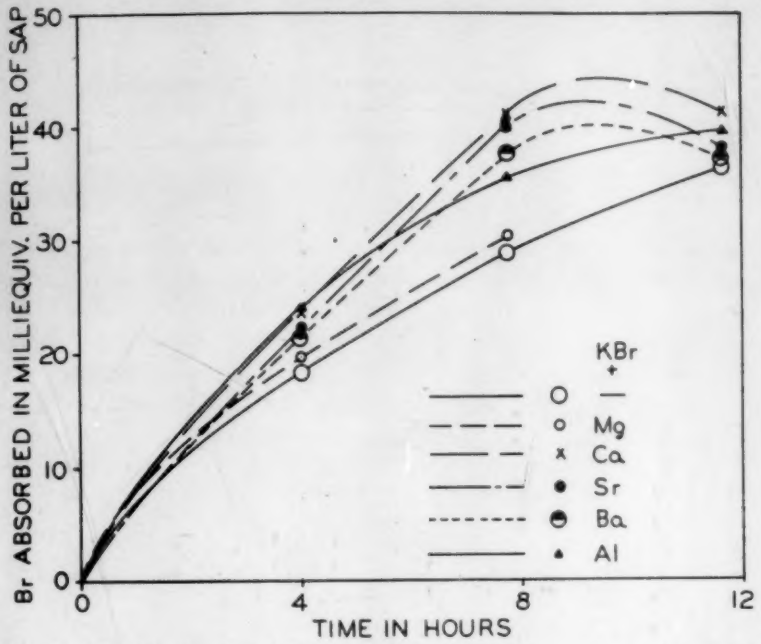

FIG. 5. Bromide absorbed in the expressed sap of barley roots treated as indicated in figure 4.

ment indicates that Ca and Mg are performing the same function and that the total increase in absorption depends upon the relative proportion and efficiency of each constituent cation.

This mutual replaceability of Ca and Mg may account for the failure of growth experiments of the type of LUNDEGARDH (**12**) to show a significant effect of Ca and Mg on K absorption. When the effect of Mg is being studied, the solution usually contains abundant Ca bringing the total divalent cation into a region on the absorption curves where the effect of increasing divalent cation has little effect. The result is that the effect of Mg as a factor in absorption may appear insignificant. The same may be true when Ca is the variable.

THE POLYVALENT CATION EFFECT AND METABOLISM

Polyvalent cations may conceivably affect either the oxidative mechanism supplying the energy for accumulation—the "pump-like" mechanism of HOAGLAND and BROYER (**8**)—or they may exert their effect primarily on the plasma membrane so that ions accomplish more readily the initial step in absorption.

Preliminary experiments indicated that there was no significant effect of Ca or Mg in the KBr solution on the rate of CO_2 production (**17**). However, MACHLIS and BROYER (unpublished data) studying the respiration of segments of primary barley root in a Fenn respirometer found a significant

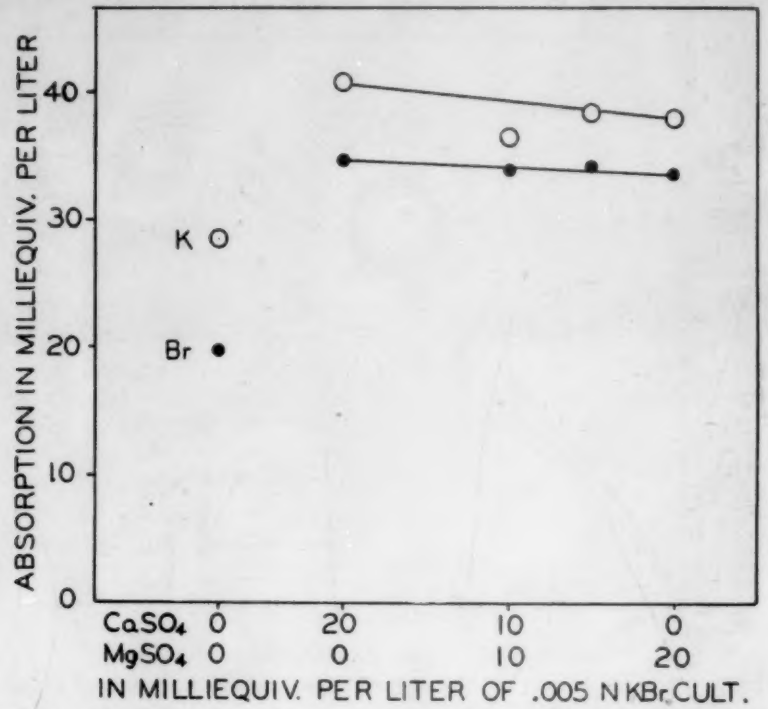

FIG. 6. Absorption of K and Br in the sap of excised roots immersed in the solutions indicated for 10 hours.

increase in both the O_2 uptake and the CO_2 respired due to the presence of Ca in the KBr absorption medium. MACHLIS also found that respiration was increased by 0.015 N $CaSO_4$ as compared with respiration of comparable root segments in distilled water. Several experiments of the writer have also shown that the increased absorption of K salts due to Ca is accompanied by a greater loss of total sugars (fig. 8). What proportion of the increased rate of respiration and sugar loss is a primary effect of Ca and how much is due to the increase in total salt absorbed is not clear. [See HOAGLAND and STEWARD (**9**) and LUNDEGARDH (**13**) for a discussion of salt absorption in relation to respiration.]

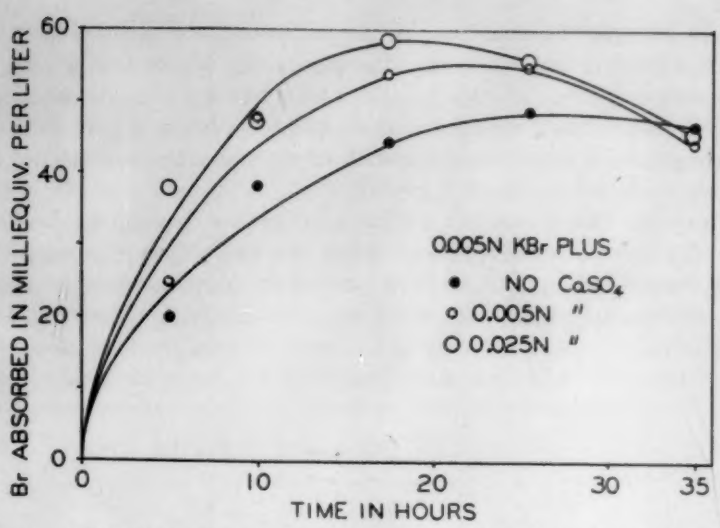

FIG. 7. Bromide absorbed in the expressed sap of barley roots immersed in the solutions indicated.

Additional evidence that Ca may increase the rate of metabolism was obtained in a time study of K and Br absorption from KBr and from KBr plus $CaSO_4$ solutions as shown in figure 7 for Br absorbed and in figure 8 for loss of total sugars. The data for K absorption were quite similar to the data for Br absorption. In figure 7 it is apparent that not only the rate but the total capacity for accumulation by excised roots is increased by the presence of Ca ions in the absorption medium.

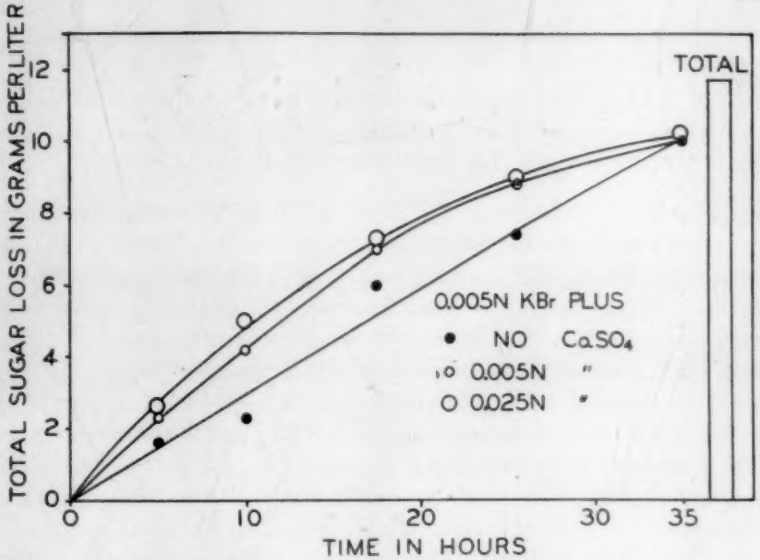

FIG. 8. Sugar loss in the expressed sap from roots immersed in the solutions indicated.

If Ca were acting as a limiting factor in metabolism, and as a result, roots responded to its presence during concurrent absorption of ions, it was conceivable that roots with higher initial Ca content at the time of the experiment would not respond to additional Ca during accumulation. In other words, the Ca effect might merely be a fortuitous result of the way in which the roots were originally grown.

To explore this possibility plants were grown during the preliminary period at three Ca levels. Subsequently the absorption of K and Br into the sap from KBr solutions and from the same solutions supplemented with $CaSO_4$ was measured. The data are shown in figure 9. The roots containing an initial supply of 4.2 m.eq. of Ca per liter were grown in the standard one-half strength HOAGLAND'S solution used for the growth of roots in all

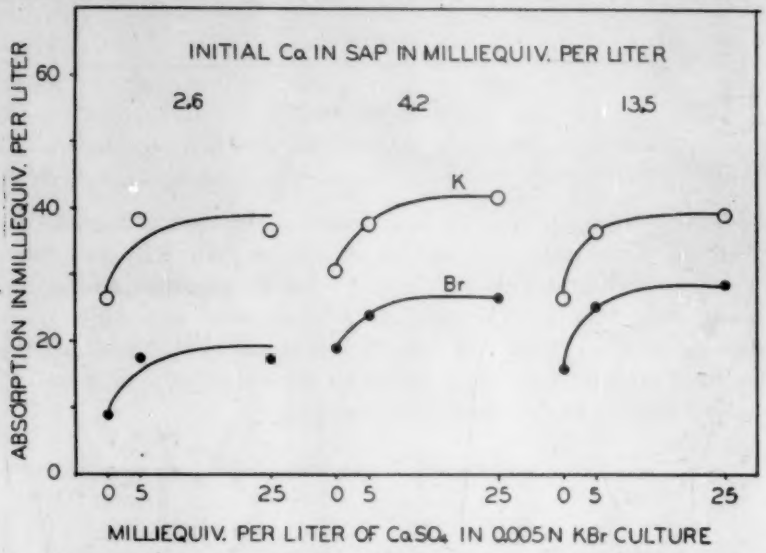

FIG. 9. Absorption of K and Br in the expressed sap of barley roots grown at various Ca levels and then placed in the solutions indicated for 12 hours.

of the foregoing experiments. The "low Ca" roots were grown concurrently in a solution containing only one-fourth as much Ca. The N as NO_3 associated with the Ca usually used in making the solutions was derived from NH_4NO_3. "High Ca" roots were grown in the standard solution almost saturated with $CaSO_4$. At the end of the growth period this solution contained a concentration of Ca twice that of the expressed sap.

Figure 9 shows that the excised roots with different Ca contents possess capacities of the same magnitude to absorb KBr from the single salt solution, and that the roots grown at all Ca levels respond to the presence of Ca in the solution during concurrent K and Br absorption. The analyses of the ashed press cakes for total K, Ca, and Br added to the calculated results for total K, Ca, and Br in the sap (cc. $\times$ m.eq./cc.) showed that the conclusions would have been substantially the same if the experiment had been appraised on the basis of the ashed, unpressed roots.

Another experiment in which the roots were excised and then placed in aerated 0.025 N $CaSO_4$ for 4 hours and then transferred to 0.005 N KBr for periods ranging from 2 to 7½ hours indicated that pretreatment with Ca had no residual effect on subsequent K and Br absorption, as compared with roots kept in aerated distilled water for the 4-hour pretreatment period.

The capacity of roots to absorb salt from KBr was to a large extent independent of the Ca status of the sap, within the limits used here; also, the roots responded almost quantitatively the same to the presence of Ca in the solutions during concurrent absorption of K and Br. These two facts may be evidence that the Ca effect is largely confined to the protoplasmic membrane and is not a direct effect on the oxidative "pump-like" mechanism. This explanation is suggested by the evidence that other ions, some of them toxic and not known to be essential for growth, are capable of performing the same function, at least to a limited extent.

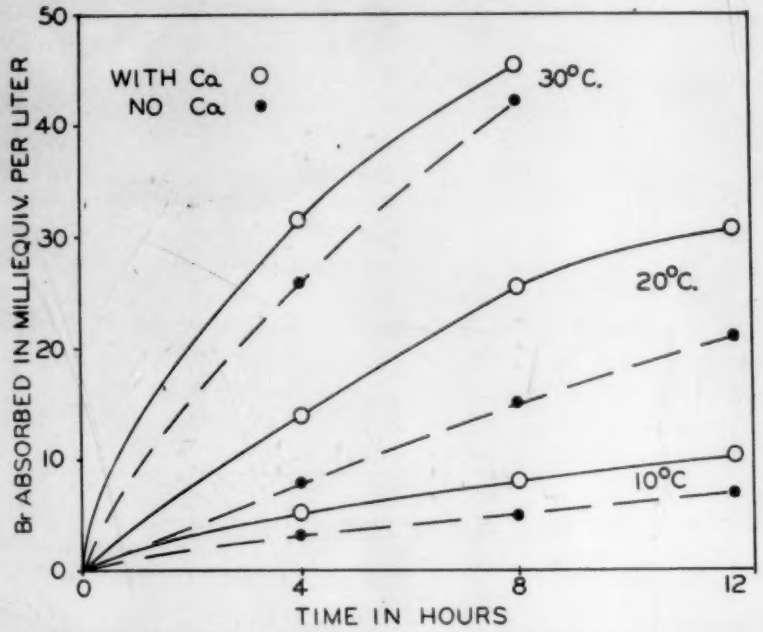

FIG. 10. Bromide absorbed in the expressed sap of excised barley roots immersed in 0.005 N KBr with and without $CaSO_4$ at the controlled temperatures indicated.

RATE AND KIND OF METABOLISM IN RELATION TO THE CA EFFECT

The experiments reported above were conducted at solution temperatures ranging from about 18° to 24° C. It was conceivable that the Ca effect was apparent at only a certain range of temperatures and that at greater extremes the influence of Ca would not be apparent. Figure 10 shows the absorption of Br in the expressed sap of roots kept for various periods in aerated 0.005 N KBr solutions with and without 0.025 N $CaSO_4$ at controlled temperatures of 10°, 20°, and 30° C. These data show that the effect

of Ca is apparent throughout a wide range of temperatures. It can be safely predicted from the results of other experiments that as the absorption temperatures approaches 0° C. the effect of Ca would disappear because the absorption of Br from 0.005 N KBr would also become negligible. The results for K absorption likewise showed the same favorable effect of $CaSO_4$ throughout this range of temperatures.

HOAGLAND and BROYER (8) have shown that active aerobic metabolism is essential for the absorption of ions even from high concentrations of KBr. Roots in which aerobic metabolism was arrested by cold, KCN, or N_2 treatment did not absorb K or Br in appreciable quantities. They interpreted

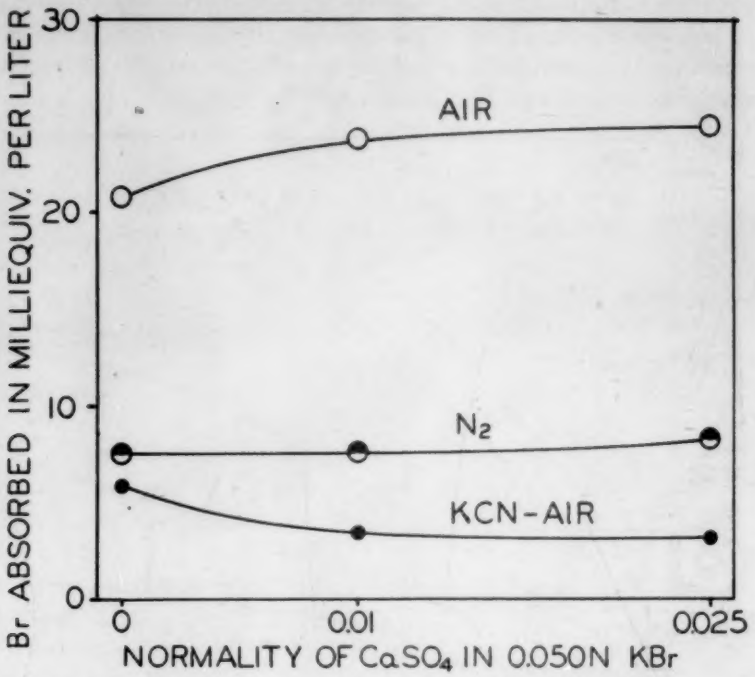

FIG. 11. Bromide absorbed in the expressed sap of roots immersed in the solutions indicated for 10 hours and aerated as noted.

this impermeability as possibly existing at the vacuolar membrane. It was therefore of interest to know if roots immersed in high concentrations of KBr with and without $CaSO_4$ would show significant differences in absorption if aerobic metabolism was arrested. Figure 11 shows the Br absorbed by roots subjected to N_2 scrubbed through alkaline pyrogallol to remove traces of oxygen and also by roots immersed in 0.005 N KCN. These results indicate that Ca does not increase the permeability of root systems to high concentrations of bromide if aerobic metabolism is arrested. The scarcely significant effect of Ca in the aerated solutions on bromide absorption may be due to the high KBr concentration used.

Discussion

Many experiments with excised barley roots have shown that Ca accelerates K and Br accumulation throughout a wide range of concentrations of both the Ca and the KBr. Al, Sr, Mg, and Ba are also able to produce the same effect, but any arrangement in a series of efficiencies depends upon the experimental conditions of time, concentration, etc. Calcium appears to be the outstanding promoter of absorption, since its effect is the greatest and is exerted over the widest range of concentrations.

The effect of polyvalent cations in the presence of KBr apparently increases the rate of respiration, the loss of sugar, and the rate and total capacity for salt accumulation. These effects may be evidence that these cations are modifying the oxidative mechanism providing the energy for accumulation.

On the other hand the wide diversity of cations, some of which are definitely toxic in growth experiments, that are able to produce the effect and that are substitutable one for the other with varying degrees of efficiency indicates that the primary effect may be either on the plasma membrane or on a surface metabolism intimately related to the permeability of the plasma membrane. Supporting evidence for this view is that roots respond to Ca during concurrent K and Br absorption regardless of their initial Ca content. Likewise, pretreatment of the roots with $CaSO_4$ for a brief period has no residual effect on K and Br absorption.

Although polyvalent cations may not effect oxidative metabolism directly, nevertheless, active aerobic metabolism is essential to show their effect. Experiments in which aerobic respiration was arrested show that Ca does not let K and Br diffuse into the roots to equal the high external concentration. The rate of aerobic metabolism, however, may vary within wide limits without affecting the magnitude of the Ca effect to an appreciable extent as shown by the temperature experiments. This dependence of the Ca effect upon aerobic metabolism does not necessarily demand that it is an effect upon the "pump-like" mechanism, but merely that it is one link in the chain of reactions transporting salt from the external medium to the vacuole.

HOAGLAND and BROYER (**8**) have suggested that the vacuolar membrane is essentially impermeable at all times to moderate diffusion gradients and that the metabolic machinery of aerobic metabolism is involved in moving ions across this membrane. The results herein are consistent with this view since Ca was not effective in increasing absorption under anaerobiosis even when K and Br could have moved along diffusion gradients if the system as a whole had been permeable.

One explanation of the data presented here is that polyvalent cations exert their effect directly upon the protoplasmic membrane. This surface effect due either to the presence of the polyvalent cation or to its absorption in the surface might conceivably be an effect on some phase of metabolism concerned with permeability [compare BLINKS, DARSIE, and SKOW (**1**)], as dissociation of membrane components, configuration of protein chains,

hydration of colloids, etc. This surface effect would demand that during rapid accumulation the resistance to ion movement offered by the protoplasmic surface is a link in relative minimum in the chain of links involved in the complex mechanism of salt accumulation from single salt solutions. Under anaerobiosis some other link in the chain becomes limiting and no effect of polyvalent cations is apparent.

LUNDEGARDH (**13**) found that mixtures of $CaCl_2$ and KCl gave more negative root potentials than did KCl alone, the decrease in potential being related to the amount of Ca in the solution. He gives as one possible explanation that Ca increases the dissociation of the membrane phosphatides, thereby giving more positions for ionic exchange. Exchange studies with radioactive isotopes in the presence and absence of Ca might confirm or deny this postulate. If Ca does increase the exchange capacity it could conceivably increase the rate of movement across the membrane because of the increase of active area. The difficulty with interpretations based on potentials is that their true relationship to absorption is not known as pointed out by HOAGLAND and STEWARD (**9**).

With the data and knowledge now at hand it is impossible to separate the effect that polyvalent cations may have on oxidative metabolism from their effects on the physical structure and metabolism of the membrane. HOAGLAND and BROYER (**8**) have directed attention to the difficulties of separating metabolism from permeability in salt accumulation by complex tissues like barley roots.

These results with excised roots are not comparable with the absorptions of plants growing in solutions or soils over extended periods of time where the rate of absorption may be modified by the availability of ions to the roots and by translocation and growth in the plant.

Summary

1. A systematic study of K and Br absorption as influenced by polyvalent cations has been made with the excised roots of barley seedlings capable of accumulating large amounts of K and Br in short periods of time.

2. Contrary to the concept of antagonism deduced from experiments conducted usually with high concentrations of salt on material incapable of active aerobic metabolism, these investigations have shown that a variety of polyvalent cations accelerate K and Br absorption, Ca being the most effective. A ratio greater than 30 Ca ions to 1 K ion in the solution was necessary before K absorption could be depressed below the absorption from pure KBr.

3. Ca, Mg, Sr, Ba, and Al ions were found to be effective in increasing the absorption of K and Br, but no regular series of efficiencies could be determined. Mixtures of $CaSO_4$ and $MgSO_4$ produced increases in absorption of the same magnitude as did these salts used alone with KBr. This indicates that the polyvalent cations are performing the same common function with different degrees of efficiency.

4. The influence of Ca on ion absorption in its relationship to the increased respiration and sugar loss is discussed and the difficulty of separating primary and secondary effects is mentioned.

5. Roots grown with different Ca supplies responded similarly to the presence of Ca in the KBr solution during subsequent ion absorption.

6. Absorption of potassium and bromide from relatively high concentrations of KBr could not be accelerated by Ca when the roots were deprived of oxygen or treated with KCN. The effect of Ca on potassium and bromide absorption is apparent over a wide range of temperatures.

7. Two interpretations of the data are discussed and the difficulties of separating metabolism from permeability in discussions of ion accumulation is reemphasized.

The writer wishes to express his appreciation to PROF. D. R. HOAGLAND for his suggestions and criticisms during the course of this research and to MR. T. C. BROYER for his suggestions and encouragement.

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THE GROWTH AND CARBOHYDRATE RESPONSES OF *AGROPYRON SMITHII* AND *BOUTELOUA GRACILIS* TO CHANGES IN NITROGEN SUPPLY

H. M. Benedict and G. B. Brown

Introduction

During recent years several papers have appeared dealing with the carbohydrate content of various grasses. Sampson and McCarty (**16**), McCarty (**8, 9**), Norman and Richardson (**15**), Morosov (**12**) and McIlvanie (**10**), have studied changes occurring during the growing season and during the transition from the vegetative to the fruitful condition of the plant. Norman (**14**), Leukel, Camp and Coleman (**5**) have in addition studied the effect of adding nitrogen to the soil on the growth and carbohydrate content of various grasses.

In the light of some of the earlier of these investigations and in connection with some work at Cheyenne, Wyoming, the question arose as to the effect of lack of nitrogen on the growth and carbohydrate content of some of the native range grasses. It was also wondered how the growth and carbohydrate content of these plants grown without nitrogen for some time would be affected when nitrogen was again made available. In this paper are presented some results obtained on the dry weight and various carbohydrates of *Agropyron smithii* Rydb. and *Bouteloua gracilis* (H.B.K.) Lag. in sand culture, as affected by removing nitrogen from the nutrient solution for a certain length of time, and then adding it again for the same length of time. These particular species were used because they are among the most commonly occurring range grasses in the central great plains.

Materials and methods

For ease in describing the procedure followed in these experiments the time during which the plants were grown can be divided into three periods. During the first period all of the plants were grown in a complete nutrient solution allowing for good growth at the start. During the second period half of the plants were grown in a nutrient solution without nitrogen, the other half in the complete or plus nitrogen nutrient solution. At the end of the second period half of the plants growing in the plus nitrogen and half of the plants in the minus nitrogen solutions were harvested and their dry weights and carbohydrate contents determined. During the third period all of the remaining plants were grown in a complete or plus nitrogen nutrient solution. At the end of the third period the plants were harvested and their dry weights and carbohydrate contents determined.

Throughout the remainder of the paper the plants grown continually in the complete or plus nitrogen solutions will be referred to as the plus nitrogen plants, while those grown in nutrient solutions without nitrogen

for a time will be referred to as the minus-nitrogen plants even though they did receive nitrogen during most of the experiment.

A comparison of the dry weights and carbohydrate contents of the plus- and minus-nitrogen plants at the end of the second period or at the time of the first harvest would indicate the effect of the lack of nitrogen on these items. The response of plants to nitrogen that had been grown without it for some time would be indicated by comparing the differences that had occurred in the plus and minus nitrogen plants during the third period or between the two harvest dates. In other words, if plants grown without nitrogen responded differently to a plus nitrogen nutrient solution than plants that had always received nitrogen, there would be an interaction between the plus- and minus-nitrogen plants and the two harvest dates. For example the sucrose content of the minus nitrogen plants might de-

TABLE I

DATES DURING WHICH PLANTS OF *Agropyron smithii* AND *Bouteloua gracilis* WERE GROWN IN NUTRIENT SOLUTIONS CONTAINING DIFFERENT AMOUNTS OF NITROGEN IN THE DIFFERENT SERIES OF EXPERIMENTS

SPECIES, YEAR AND ORIGIN	TREATMENTS					
	ALL PLANTS IN A COMPLETE OR PLUS-NITROGEN SOLUTION		HALF OF PLANTS IN A COMPLETE NITROGEN SOLUTION AND HALF IN A MINUS-NITROGEN SOLUTION		ALL PLANTS IN A COMPLETE OR PLUS-NITROGEN SOLUTION	
Agropyron smithii						
1936 seed	5/28	7/9	7/9	8/21	8/21	10/1
1938 clones 1 yr. old	6/16	7/30	7/30	9/12	9/12	10/26
1939 clones 2 yr. old	7/1	8/1	8/1	9/1	9/1	10/1
Bouteloua gracilis						
1936 seed	5/28	7/9	7/9	8/21	8/21	10/1
1938 clones 1 yr. old	6/16	7/30	7/30	9/12	9/12	10/26
1940 clones 3 yr. old	6/1	7/1	7/1	8/1	8/1	9/1

crease between the harvest dates while that of the plus nitrogen plants might increase.

Three series of experiments were carried out on both species. Plants of *Agropyron smithii* were grown from seed in 1936, from one year old clones in 1938, and from two year old clones in 1939. Plants of *Bouteloua gracilis* were grown from seed in 1936, from one year old clones in 1938, and from three year old clones in 1940. The dates on which the plantings were made, the duration of the periods, and thus, the harvesting dates in the various years, are shown in table I. It can be seen that the procedure outlined above was followed every year, but that the dates of starting the experiments, the duration of the periods, and the harvest dates, varied from year to year.

The plants were grown in two-gallon glazed crocks or in asphalt coated galvanized iron tanks $1' \times 2' \times 1\frac{1}{2}'$, filled with quartz sand to which nutrient solutions were applied by the hand operated system described by WITHROW

and BIEBEL (**19**). In 1936 when the experimental plants were started from seed, 25 plants were grown in each crock. In starting the plants from clones, sections of the sod of the desired species were dug up from the grass nursery. The tops were clipped off and the sod then dissected into small portions of equal fresh weights of 2 grams each. In 1938 fifteen of these portions were planted to a tank and in 1939 and 1940, five were planted to a 2-gallon crock. This insured that every plant in each experiment developed from approximately the same amount of material. The composition of the nutrient solutions are shown in table II. The complete or plus nitrogen solution is the one used by WERNER (**18**). Iron was added at the rate of 1 ml. of a 5 per cent. solution of ferric citrate per liter of nutrient solution. The nutrient solutions were made up with tap water which supplied the other minor elements. The pH of the solutions was maintained at about 6.0.

In harvesting, the plants were carefully washed free of sand, dried between toweling paper and the fresh weights of the roots and tops determined. The plants were then cut into pieces ¼ inch long and weighed samples taken for dry weight determinations. These samples were dried to a constant weight at 100° C. the percentage dry weight determined, and the dry weight per plant calculated.

TABLE II

MOLAR CONCENTRATION OF NUTRIENT SOLUTIONS

SALTS USED	PLUS NITROGEN OR COMPLETE	MINUS NITROGEN
KH_2PO_4	0.0045	0.0045
$Ca(NO_3)_2$	0.0060	.....
$(NH_4)_2SO_4$	0.0030	.....
$MgSO_4$	0.0045	0.0045
$CaCl_2$	.....	0.0060
Na_2SO_4	.....	0.0030

Aliquots were also taken of the cut-up plants, placed in boiling 95 per cent. ethyl alcohol for 20 minutes, sealed and set aside until needed for carbohydrate determinations. The carbohydrate fractions determined were reducing sugars, sucrose, starch, and hemicelluloses. It should be mentioned that the above names are used as a convenience in referring to the fraction obtained by the method of analysis used. Thus, the carbohydrate referred to as starch in this paper, will include the materials hydrolyzed by Taka diastase, following the removal of the alcohol-soluble compounds. The hemicelluloses will include the compounds hydrolyzed by 1 per cent. HCl following the removal of the diastase hydrolyzable materials.

The sugars were extracted with alcohol in a Landseidl apparatus and the alcohol boiled off under reduced pressure. The reducing sugars were determined by the QUISUMBING and THOMAS reduction method and the BERTRAND copper determination method in 1936. In the later series the modification of the LANE-EYNON method described by HILDRETH and BROWN (**3**) was

used. Sucrose was determined by inverting a 50-ml. aliquot of the sugar solution according to the method described by HILDRETH and BROWN (3) determining the reducing sugars in the aliquot of this inverted solution by the methods used for reducing sugars, subtracting the amount of reducing sugar from this and multiplying by 0.95.

Starch and hemicelluloses were determined by the method outlined by JOHNSTON and DORE (4) except that the copper reductions and determinations were carried out by the QUISUMBING and THOMAS and BERTRAND methods in 1936 and later by the LANE-EYNON method as modified by HILDRETH and BROWN (3).

The various treatments were always randomized among themselves on the greenhouse bench to insure as far as possible that the plus- and minus-nitrogen plants were subjected to identical conditions with the exception of the presence or absence of nitrogen in the nutrient solutions. In 1939 and 1940 sufficient crocks were planted to allow for dry weight and carbohydrate determinations on 8 replications of each treatment on both harvest dates. As the replications were randomized, analyses of variance as described by SNEDECOR (17) were run on the results of the various determinations. If the differences due to treatments, or to the interaction treatment by dates were found to be significant, the differences required for significance are given with the results. A difference was considered significant if the odds against it occurring by chance were greater than 19 to 1.

Results

In the tables presenting the results the first value given under each year is that obtained at the first harvest date or at the end of the second period, while the second value given is that obtained at the second harvest date or at the end of the third period. The values given for the plants grown in 1936 are the means of two replications, 25 plants to a replication; those for plants grown in 1938 are the means of 15 plants grown in a single culture; and those for plants grown in 1939 and 1940 are the means of 8 replications, 5 plants to a replication. It should also be mentioned that the required values for significance, between means, and for an interaction apply to 1939 and 1940 data only.

The dry weights per plant and the top-root ratios of *Agropyron smithii* are shown in table III. In two of the three years the roots of the minus-nitrogen plants had a greater dry weight than those of the plus-nitrogen plants at the time of the first harvest, but had a smaller dry weight in all three years at the date of the second harvest, or at the end of the third period. The dry weights of the tops and the total plants were always greater in the plus-nitrogen plants. In all three years the top-root ratios of the minus-nitrogen plants were always smaller than those of the plus-nitrogen plants. Between the two harvest dates, however, the ratios of the plus-nitrogen plants increased while those of the minus-nitrogen plants decreased.

Table IV gives the dry weights and top-root ratios of the plants of *Bouteloua gracilis*. The dry weights of the roots of the minus-nitrogen plants in 1936 were greater than those of the plus-nitrogen plants, but in 1938 and 1940 there was little difference between them at the first harvest date. At the date of the second harvest the roots of the plus-nitrogen plants weighed more than those of the minus-nitrogen plants except in 1940. The dry weights of the tops and the entire plants with the exception of those at the second harvest date in 1940 were always greater in the plus-nitrogen

TABLE III

DRY WEIGHT PER PLANT AND TOP-ROOT RATIOS OF *Agropyron smithii* WHEN GROWN IN PLUS-NITROGEN AND MINUS-NITROGEN NUTRIENT SOLUTIONS

PLANT PART AND TREATMENT	YEAR						DIFFERENCE REQUIRED FOR SIGNIFICANCE BETWEEN MEANS*
	1936		1938		1939		
	DATE OF HARVEST						
	8/21	10/1	9/12	10/26	9/1	10/1	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Roots							
Plus N†	0.165	0.889	2.045	14.390	0.702	1.994	
Minus N‡	0.239	0.639	1.325	6.804	0.960	1.244	0.288
Tops							
Plus N	0.847	3.245	5.765	18.144	1.626	3.213	
Minus N	0.356	1.112	2.336	8.270	0.869	1.601	0.328
Total plant							
Plus N	1.102	4.134	7.810	32.524	2.328	5.207	
Minus N	0.595	1.751	3.661	15.074	1.829	2.845	0.636
	ratio	*ratio*	*ratio*	*ratio*	*ratio*	*ratio*	*ratio*
Tops ÷ roots							
Plus N	5.13	3.65	2.82	1.76	2.28	1.61	
Minus N	1.49	1.74	1.26	1.22	0.89	1.29	0.32

* If values differ by more than the figure given in this column the odds are greater than 19 to 1 that the difference was not due to chance. This applies to 1939 data only.

† These plants were grown in a complete nutrient solution throughout the experiment.

‡ These plants were grown for a time previous to the first harvest date in a nutrient solution lacking nitrogen, but in a complete nutrient solution from the first to the second harvest date.

than in the minus-nitrogen plants. The top-root ratios of the minus-nitrogen plants, with one exception were always less than those of the plus-nitrogen plants. The ratios of the minus-nitrogen plants always increased markedly between harvest dates while those of the plus-nitrogen plants remained approximately the same.

The percentages of the various carbohydrates present in the plants of *Agropyron smithii* are shown in table V. The highest percentages of reducing sugar, sucrose, and starch were always found in the minus-nitrogen plants at the first harvest date immediately following the period during which they had received no nitrogen. The percentages of these three materials decreased more in the minus-nitrogen than in the plus-nitrogen plants between the two harvest dates.

The hemicellulose contents of the plus- and minus-nitrogen plants never differed greatly from each other and they usually varied in the same direction between the first and second harvest. The statistical analyses showed a significant interaction between treatments and dates, with the exception of the hemicelluloses in the roots and the tops and the reducing sugars in the tops.

The carbohydrate content of the plants of *Bouteloua gracilis* are shown in table VI. The reducing sugar content of the plants did not differ greatly

TABLE IV

DRY WEIGHT PER PLANT AND TOP-ROOT RATIOS OF *Bouteloua gracilis* GROWN IN PLUS- AND MINUS-NITROGEN NUTRIENT SOLUTIONS

PLANT PART AND TREATMENT	YEAR						DIFFERENCE REQUIRED FOR SIGNIFICANCE BETWEEN MEANS*
	1936		1938		1940		
	DATE OF HARVEST						
	8/21	10/1	9/12	10/26	8/1	9/1	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Roots							
Plus N†	0.351	0.715	1.552	3.498	0.651	1.110	
Minus N‡	0.511	0.680	1.526	1.950	0.639	1.407	0.243
Tops							
Plus N	1.633	3.329	5.143	12.513	3.488	6.216	
Minus N	0.966	1.689	3.651	8.799	2.133	6.244	0.793
Total plant							
Plus N	1.983	4.044	6.695	16.011	4.139	7.326	
Minus N	1.477	2.378	5.177	10.749	2.772	7.651	1.210
	ratio	*ratio*	*ratio*	*ratio*	*ratio*	*ratio*	*ratio*
Tops ÷ roots							
Plus N	4.65	4.65	3.31	3.57	5.03	5.56	
Minus N	1.89	2.49	2.39	4.50	3.21	4.44	0.80

* If values differ by more than the figure given in this column the odds are greater than 19 to 1 that the difference was not due to chance. This applies to 1940 data only.

† See footnote †, table III.

‡ See footnote ‡, table III.

between treatments and dates but the highest percentage was usually found in the minus-nitrogen plants at date of the first harvest. The percentage of sucrose in both the roots and tops was generally higher in the minus-nitrogen than in the plus-nitrogen plants. Between harvest dates the roots of the minus-nitrogen plants showed a greater decrease in sucrose than did the roots of the plus-nitrogen plants. With the exception of the 1936 series the sucrose content of the tops of both the plus- and minus-nitrogen plants decreased between the first and second harvestings. The starch content of the tops of the minus-nitrogen plants was usually greater than that of the plus-nitrogen plants but the reverse was true of the roots. The starch content of the roots of both sets of plants usually increased between harvest dates while that of the tops usually decreased. Two exceptions were the roots of the plus-nitrogen plants in 1940 and the tops of the plus-nitrogen

TABLE V

CARBOHYDRATE CONTENT OF PLANTS OF *Agropyron smithii* GROWN IN PLUS- AND MINUS-NITROGEN NUTRIENT SOLUTION

TREATMENT	YEAR						DIFFERENCE REQUIRED FOR SIGNIFICANCE*	
	1936		1938		1939			
	DATE OF HARVEST							
	8/21	10/1	9/12	10/26	9/1	10/1		
	%	%	%	%	%	%		%
Reducing sugars								
Roots								
Plus N†	2.12	0.38	1.79	0.72	2.31	2.13	Between means	0.42
Minus N‡	2.22	0.88	1.93	0.73	2.96	1.80	For interaction	0.59
Tops								
Plus N	0.87	0.94	1.10	1.08	1.29	1.10	Between means	0.17
Minus N	1.40	0.95	1.86	1.05	1.39	1.15	For interaction	Not sig.
Sucrose								
Roots								
Plus N	2.30	3.41	2.18	1.41	3.50	4.12	Between means	1.32
Minus N	11.26	1.83	12.33	0.85	11.86	3.00	For interaction	1.87
Tops								
Plus N	2.16	3.31	1.70	1.58	2.50	2.92	Between means	0.74
Minus N	3.15	2.50	3.54	1.42	3.94	3.08	For interaction	1.05
Starch§								
Roots								
Plus N	8.06	15.28	5.09	2.33	2.85	3.56	Between means	0.74
Minus N	16.95	14.93	6.44	2.19	3.80	2.63	For interaction	1.05
Tops								
Plus N	7.65	11.72	1.94	1.91	1.69	2.13	Between means	0.93
Minus N	11.63	11.17	8.01	1.05	4.30	1.53	For interaction	1.32
Hemicelluloses								
Roots								
Plus N	3.76	3.93	16.94	14.56	19.39	24.12	Between means	1.78
Minus N	3.43	4.38	13.38	11.11	18.11	21.83	For interaction	Not sig.
Tops								
Plus N	3.29	3.96	16.99	15.63	22.04	19.28	Between means	Not sig.
Minus N	6.32	4.24	17.21	20.03	21.14	18.99	For interaction	Not sig.

* See footnote *, table III.
† See footnote †, table III.
‡ See footnote ‡, table III.
§ Substances hydrolyzed by Taka diastase.

TABLE VI

THE CARBOHYDRATE CONTENT OF PLANTS OF *Bouteloua gracilis* GROWN IN PLUS- AND MINUS-NITROGEN NUTRIENT SOLUTIONS

TREATMENT	YEAR						DIFFERENCE REQUIRED FOR SIGNIFICANCE*	
	1936		1938		1940			
	DATE OF HARVEST							
	8/21	10/1	9/12	10/26	8/1	9/1		
	%	%	%	%	%	%		%
Reducing sugars								
Roots								
Plus N†	1.08	0.64	1.13	1.22	1.43	2.01	Between means	Not sig.
Minus N‡	1.80	0.66	1.19	0.82	1.79	1.81	For interaction	Not sig.
Tops								
Plus N	0.99	0.98	1.55	0.89	1.44	1.99	Between means	0.25
Minus N	1.17	1.08	1.28	0.67	1.65	1.85	For interaction	Not sig.
Sucrose								
Roots								
Plus N	1.68	3.41	2.42	0.98	1.74	2.65	Between means	0.90
Minus N	3.80	4.10	3.17	0.90	4.94	2.79	For interaction	1.27
Tops								
Plus N	1.54	1.83	1.70	0.57	2.97	2.02	Between means	0.81
Minus N	2.56	1.87	2.48	0.92	3.90	2.56	For interaction	Not sig.
Starch§								
Roots								
Plus N	5.41	13.22	2.34	3.86	1.49	0.67	Between means	0.96
Minus N	4.66	15.13	1.94	2.12	0.84	2.69	For interaction	Not sig.
Tops								
Plus N	5.61	8.83	1.92	1.21	1.00	0.86	Between means	Not sig.
Minus N	10.67	8.40	2.44	1.96	1.24	1.60	For interaction	Not sig.
Hemicelluloses								
Roots								
Plus N	3.70	3.16	10.21	6.33	27.14	26.12	Between means	Not sig.
Minus N	3.72	3.65	9.02	5.04	25.45	26.53	For interaction	Not sig.
Tops								
Plus N	5.27	3.94	12.84	19.64	22.16	26.20	Between means	Not sig.
Minus N	4.57	3.20	19.78	23.21	24.70	24.00	For interaction	Not sig.

* See footnote *, table IV.
† See footnote †, table III.
‡ See footnote ‡, table III.
§ Substances hydrolyzed by Taka diastase.

plants in 1936. The hemicellulose content did not vary greatly between treatments and dates, and in general the percentage in both the plus- and minus-nitrogen plants varied in the same direction between the two dates of harvesting. The statistical analyses showed a significant interaction between treatments and dates only in the case of the non-reducing sugar content of the roots.

Discussion

In discussing the results, the dry weight determination will be considered first, followed by the carbohydrate analyses.

The effect of the different amounts of available nitrogen on the growth of the plants of both species as measured by their dry weights were what were to be expected and agree in general with the results of HARRISON (**2**) on *Poa pratensis*. The results presented here indicate that under the conditions of these experiments lack of nitrogen retarded the total growth of plants of *Agropyron smithii* and *Bouteloua gracilis* but tended to favor the growth of the roots as compared to the tops of the plants; while abundant nitrogen tended to favor the growth of the tops as compared to the growth of the roots. This was shown by the fact that the total dry weights of the plants that had been grown without nitrogen for a time were always less than the total dry weights of the plants that had always received nitrogen; while the top-root ratios of the former plants were always smaller than those of the latter. The increase in the top-root ratios on the addition of nitrogen to plants that had been without it for a time, under conditions which resulted in an increase in these ratios in plants of *Agropyron smithii* and practically no change in the ratios in plants of *Bouteloua gracilis* that had always received nitrogen, indicate further that an abundant nitrogen supply favored the top growth while lack of it favored root growth in these two species.

The proportionate increases in total dry weights of plants of *Agropyron smithii* between harvest dates as given by dividing the weight at the date of the second harvest by that at the first harvest date were for the plus-nitrogen plants 3.75, 4.54, and 2.24 and for the minus-nitrogen plants 2.94, 4.12, and 1.55 in 1936, 1938, and 1939, respectively. The proportions for the plus-nitrogen plants of *Bouteloua gracilis* were 2.04, 2.39, and 1.77 and for the minus-nitrogen plants were 1.61, 2.07, and 2.76, respectively, for the years 1936, 1938, and 1940. These ratios indicate that under the conditions of these experiments the growth rate of *Agropyron smithii* when slowed by lack of nitrogen was not immediately increased on the addition of nitrogen to that of plants that had not suffered from nitrogen deficiency. For *Bouteloua gracilis* these ratios indicate that under certain conditions the retarding of the growth rate due to lack of nitrogen was completely overcome when nitrogen was again made available. This is further indicated for *Bouteloua gracilis* by the lack of a significant difference between the total dry weights of the plus- and minus-nitrogen plants at the end of the 1940 experiments.

In discussing the results on the carbohydrates the results obtained on the first harvest date or at the end of the period during which the minus-nitrogen

plants were grown in the absence of nitrogen will be considered first. On this date in all three years the sucrose and starch content of *Agropyron smithii* was always much higher in the minus- than in the plus-nitrogen plants. The greatest increase in sucrose content occurred in the roots, while that of the starch content occurred in the tops, except in 1936. Likewise on this harvest date the minus-nitrogen plants of *Bouteloua gracilis* always had a higher percentage of sucrose than the plus-nitrogen plants. This was true of both the roots and the tops, but the difference was greater in the roots. There was no consistent difference between the plus- and minus-nitrogen plants as far as the starch content of *Bouteloua gracilis* and the reducing sugar and hemicellulose content of both species was concerned. Thus it seemed that the principal effect on the carbohydrate content of withholding nitrogen from plants of *Agropyron smithii* was to increase the sucrose and starch content of both the roots and the tops with the greatest increase of sucrose occurring in the roots and of starch occurring in the tops. In the case of *Bouteloua gracilis* lack of nitrogen tended to increase the sucrose content especially of the roots.

Between the harvest dates or during the period nitrogen was again made available to the minus-nitrogen plants, the sucrose and starch content of the minus-nitrogen plants of *Agropyron smithii* and the sucrose content of the minus-nitrogen plants of *Bouteloua gracilis* always decreased, and at least in 1939 and 1940 by a highly significant amount. While these compounds were decreasing in the minus-nitrogen plants, in two of the three years they were increasing in the plus-nitrogen plants. In the other year, 1938, the percentage of these compounds in the minus-nitrogen plants decreased a great deal more than in the plus-nitrogen plants. Thus, under conditions of temperature, day length, etc., which favored the building up of sucrose and starch in plants that had been grown continually in the presence of nitrogen, these compounds decreased in plants which had been without nitrogen for a time. This interaction between the plus- and minus-nitrogen plants and the two harvest dates which was consistently found for the sucrose and starch content of *Agropyron smithii* and the sucrose content of *Bouteloua gracilis* was not generally found for the other carbohydrates determined. These results indicated that under the conditions of these experiments making nitrogen available to plants that had been without it for a while resulted in a decrease in the sucrose and starch content of *Agropyron smithii* and the sucrose content of *Bouteloua gracilis*, without having much effect on the reducing sugar and hemicellulose content of both species and on the starch content of *Bouteloua gracilis*.

It is generally considered that withholding nitrogen from plants tends to build up their carbohydrate reserves, and that these reserves are utilized by the plant for renewed growth when nitrogen again becomes available and other conditions for growth are favorable. The increase in the percentage of sucrose in *Agropyron smithii* and *Bouteloua gracilis* and of starch in *Agropyron smithii* when nitrogen was lacking and the decrease in the per-

centage of these compounds when nitrogen was again made available indicated that these compounds were serving as carbohydrate reserves. It is possible, however, for the percentage of a compound in a plant to decrease, yet because of the increase in dry weight, for the number of grams in the plant to increase. Thus, to be certain that these plants were using the above compounds as reserve carbohydrates it was necessary to show a decrease in the number of grams per plant on the addition of nitrogen. In the 1939 series, each plant of *Agropyron smithii* grown without nitrogen contained 148 milligrams of sucrose and 74 milligrams of starch. After being grown in the presence of nitrogen for one month each plant contained 84 milligrams of sucrose and 57 milligrams of starch, a loss of 64 milligrams of sucrose and 17 milligrams of starch. In 1940 the sucrose content of the minus-nitrogen plants of *Bouteloua gracilis* decreased in percentage, but increased from 125 to 199 milligrams per plant after nitrogen had been made available for a month. These plants, however, increased in size very rapidly during this period and it may be that by the end of the month the new growth had practically ceased and had begun to replenish the carbohydrates used in its initiation. If the analyses had been made two weeks after the addition of nitrogen the number of milligrams per plant might have been less than it was at the time of the first harvest. These data on the milligrams of materials per plant gave no definite indication as to whether or not sucrose was used by *Bouteloua gracilis* as a reserve, but they showed fairly conclusively that under the conditions of these experiments both sucrose and starch served as reserve carbohydrates in plants of *Agropyron smithii*.

McCarty (**8**) and others have shown that a plant in the vegetative stage of its life cycle may differ in its carbohydrate content from a plant in the fruiting stage, and it might be wondered if the stage of the life cycle of the plants under consideration in this paper had any effect on their carbohydrate content. The plants of *Bouteloua gracilis* in all three years were all in flower before the different treatments were started and continued to flower in all the treatments throughout the experiments. None of the plants of *Agropyron smithii* ever flowered regardless of the treatment. Therefore, as both species remained in the same stage of their life cycle throughout the experiments, the changes in carbohydrate content cannot be ascribed to a change in the stage of the plant's life history.

It is generally conceded that starch as such is not found in the vegetative parts of grasses. Loomis (**6**) states "in common with most grasses starch is not found in the vegetative parts of corn." Norman (**14, 15**) makes no mention of starch in the vegetative parts of *Lolium perenne* and *Dactylis glomerata*. According to Loomis and Shull (**7**) Taka diastase will hydrolyze dextrine and other compounds along with starch to copper reducing substances. Tests with iodine on thin sections have shown, especially in the parenchyma of the roots of the minus-nitrogen plants, minute granules which stained a lavender or purple with iodine. Different observers of these

granules have not been sure that these were starch granules since the color with iodine was not quite right nor were concentric rings apparent. Thus, it may be doubted that the material hydrolyzed by Taka diastase and reported here as starch was really such. NORMAN (**14, 15**) has shown that fructosans form reserves in *Dactylis glomerata* and *Lolium perenne*, and MOLISCH (**11**) reports polysaccharides of fructose in many grasses including *Agropyron repens*. It may be that the materials reported as starch in this paper were at least in part fructosans, since these substances are found in one closely related species, *Agropyron repens*, and since according to ARCHBOLD (**1**), KIHARA reporting in the Journal of the Agricultural Chemical Society of Japan found that fructosans of several species of *Allium* were hydrolyzed by Taka diastase.

In these experiments the plants of *Agropyron smithii* were started from seed, from clones 1 year old, and from clones 2 years old; the plants of *Bouteloua gracilis* were started from seed, from clones 1 year old, and from clones 3 years old. It is interesting to note that the older the source from which the plants originated the higher the percentage of hemicelluloses and the lower the percentage of starch present. This suggests that these changes in percentage were perhaps associated with the senility of these plants. It might be argued that this increase in hemicellulose and decrease in starch was due to the conditions existing in the different years in which the experiments were carried on. This of course is a possibility but it does not seem likely that the conditions in a greenhouse with plants grown in nutrient solutions would become increasingly favorable from 1936 to 1940 for hemicellulose formation and increasingly unfavorable for starch formation.

Summary and conclusions

1. Plants of *Agropyron smithii* and *Bouteloua gracilis* were grown from seed and from clones in three different years in complete and minus-nitrogen nutrient solutions.

2. The dry weights of the roots, tops, entire plants, top-root ratios, and the reducing sugar, non-reducing sugar, starch (Taka diastase hydrolyzable material) and hemicellulose contents of the plants of both species were determined.

3. The results of the dry weight and top-root ratio determinations showed that lack of nitrogen reduced the total dry weight of the plants of both species but favored the growth of the roots as compared to that of the tops, while abundant nitrogen favored the growth of the tops as compared to the roots.

4. The results of the carbohydrate analyses showed that the principal effect of the lack of nitrogen was to increase the percentage of sucrose and starch in the plants of *Agropyron smithii* and of sucrose in the plants of *Bouteloua gracilis*. They also showed that making nitrogen available to plants that had been without it for a time tended to reduce the percentage of the carbohydrates built up when nitrogen was lacking.

5. Calculations from the percentages showed that the number of milligrams of sucrose and starch per plant of *Agropyron smithii* decreased when nitrogen was made available to plants that had been without it for a while. This indicated that these two substances served as carbohydrate reserves in the plants of this species.

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INFLUENCE OF PHOSPHORUS SUPPLY AND THE FORM OF AVAILABLE NITROGEN ON THE ABSORPTION AND THE DISTRIBUTION OF PHOSPHORUS BY THE TOMATO PLANT[1]

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(WITH TEN FIGURES)

Introduction

In the course of studies on the rôle of phosphorus in the synthesis of proteins by the tomato plant it was noticed that the form in which nitrogen was supplied to the plants had a distinct effect on the development of deficiency symptoms due to lack of phosphorus. A similar experiment has been reported by Skok (**9**), who found that the appearance of calcium deficiency symptoms in the bean plant was noticeably delayed in plants that received urea as a source of nitrogen as compared with those that received nitrate nitrogen. He interpreted these results to be in support of Eckerson's findings (**7**) that calcium is essential for the assimilation of nitrates, by being necessary for the successful catalysis of the reduction of nitrates to nitrites by the so-called reducase enzyme. His plants receiving urea were thus supplied with a reduced form of nitrogen and the necessity for nitrate reduction was eliminated. Skok based his conclusions entirely upon fresh and dry weight data and appearance of the plants. He suggested that since other elements (potassium, phosphorus, and sulphur) were also found to be necessary for normal reducase activity by Eckerson (**7**), their deficiency symptoms may also be lessened in severity by the use of urea.

Preliminary experiments in this laboratory indicated clearly that tomato plants utilizing urea as a source of nitrogen, when deprived of their normal supply of phosphorus, did not exhibit the usual signs of phosphorus deficiency as soon as did deficient plants growing in a medium containing nitrate nitrogen. The question arose as to whether this was due to non-utilization of nitrates by the plants receiving nitrate nitrogen, as suggested by Eckerson, or to some other cause. Several experiments in which the plant tissue was dried and analyzed for phosphorus showed clearly that the plants utilizing urea contained much more phosphorus per unit of dry weight than did the plants receiving nitrate nitrogen. Furthermore, during phosphorus starvation it took the plants growing in a medium containing urea longer to deplete this reserve store of phosphorus. It thus became evident that the delay in the appearance of deficiency symptoms by the plants furnished urea might be due to a greater accumulation of phosphorus and not to the removal of the necessity for nitrate reduction by the substitution of urea for nitrate in the nutrient solution.

[1] Journal Paper no. 139 of the Purdue Agricultural Experiment Station.

In the present experiments an attempt was made to study this problem further by measuring the rate of uptake of phosphorus by normal and phosphorus-deficient plants growing in media supplying urea and nitrate nitrogen. The use of radiophosphorus has proven quite convenient in studies of similar nature (**1, 2, 10**), and was employed in the present experiments as an indicator by which the concentration of newly absorbed phosphorus was measured. Also reported is an experiment designed to compare the distribution of newly absorbed phosphorus in phosphorus deficient and normal plants.

Materials and methods

The composition of the nutrient solutions used in these experiments is given in table I. In addition, the solutions contained the following micro-

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS*

COMPOUND	NITRATE PLUS PHOSPHORUS	NITRATE MINUS PHOSPHORUS	UREA PLUS PHOSPHORUS	UREA MINUS PHOSPHORUS
KCl	0.005	0.006	0.001	0.002
$MgSO_4$	0.002	0.002	0.002	0.002
$Ca(NO_3)_2$	0.005	0.005	.....	.....
KH_2PO_4	0.001	.....	0.001	.....
$CO(NH_2)_2$	.....	.....	0.005	0.005
$CaCl_2$	.....	.....	0.005	0.005
K_2SO_4	.....	.....	0.002	0.002

* Figures represent molarity.

elements: boron, 0.5 p.p.m. as H_3BO_3; manganese, 0.5 p.p.m. as manganese sulphate; zinc, 0.5 p.p.m. as zinc sulphate; copper, 0.125 p.p.m. as copper sulphate; and iron, 1.0 p.p.m. as iron sulphate.

While the plants were growing in sand, the pots were flushed daily with nutrient solution to wash away accumulated salts and provide a medium of as nearly constant acidity as possible.

Tomato seeds (variety, Indiana Baltimore) were sown in quartz sand. When the seedlings were about 3 inches in height, they were transplanted to half-gallon glazed crocks filled with fresh quartz sand. The drainage hole was covered with glass wool to allow free drainage. The plants were grown approximately three weeks, until about 8 to 10 inches in height, before removing the phosphorus from one lot receiving nitrate nitrogen and one lot receiving urea. Four treatments were employed: nitrate plus phosphorus, nitrate with no phosphorus, urea plus phosphorus, and urea with no phosphorus. Ten plants from each group were used for actual experiments but usually about 20 plants per treatment were available and the plants were selected for the absorption experiments on the basis of uniformity of size and appearance.

When the plants were about eight weeks old, they were transferred to two-quart Mason jars filled with nutrient solution containing known amounts

of phosphorus and radiophosphorus. The jars were painted black to exclude light, and the solutions were aerated continuously through sintered glass aerators connected to a central compressor. The importance of adequate aeration and pH control in studies of this type has been emphasized by many investigators. Aeration provided by the sintered glass outlets used in this work was quite uniform and certainly adequate. In most cases the pH of the nutrient solution was kept at 4.8 to 5.0. In a second experiment part of the series of plants furnished urea were grown at pH 6.8 to 7.0 (figs. 4, 5, 6). This was done by the addition of normal potassium hydroxide solution to the nutrient solution.

After the plants were transferred to the culture jars, no attempt was made to adjust the acidity of the solutions during the period of absorption. The time interval of the absorption experiments was four days; and every ten hours one plant was removed from each treatment and divided into leaves, stems, and roots. The samples were dried at 80° C. in a forced draft air oven overnight and then ground to a powder in a Wiley mill. One gram (less if necessary) of the well-mixed material was weighed into a porcelain crucible and ashed at 550° C. for four hours. The radioactivity of each sample was determined by counting on a GEIGER-MÜLLER counter, the counts were corrected for the background count of the counter and for radioactive decay, and calculated on the basis of the number of counts per minute per gram of dried material.

The original sample of radioactive phosphorus used in all the experiments was in solution as disodium phosphate and consisted of 49.5 mg. of disodium phosphate having a total activity of 2.00 millicuries. Since the radioactivity of the sample was known, it was possible to calculate the correct amount to use per plant so that the plant samples would come within the range of the counter. One tenth ml. of the original solution was diluted to one liter with distilled water and the correct amount of this solution supplied to each plant. This solution containing P^* (radiophosphorus atom) was measured into each jar and then 1850 ml. of nutrient solution which in all cases contained the normal amount of phosphorus was added. One-ml. samples were withdrawn from the jars as soon as each lot was filled. These were placed in crucibles, dried, heated in the muffle furnace, and counted for comparison samples to get an actual measure of the radioactivity of the culture solutions. Ten-ml. samples were withdrawn from each culture solution at the time of sampling, dried, ashed, and counted. This was done to get an independent check on the amount of phosphorus removed from the culture solution by each plant.

In arriving at the final activities of the ashed samples, a series of ten one-minute counts was made on each sample. The mean error and the mean were calculated for each sample. The mean value was then corrected for background count, radioactive decay, and comparison sample of the nutrient solution, and was finally calculated in terms of $P^*O_4^{\equiv} \times 10^3$ expressed as p.p.m. of dry matter. This procedure gave results which are convenient for

plotting. It is to be emphasized, however, that these values are relative, depending on the strength and volume of the nutrient solution, and have no more actual significance than counts per gram of dry weight. The methods of calculation used in this work have been described by others (**8**) and need not be discussed here.

The plan of the third experiment was to separate the phosphorus compounds of the plant into different fractions by solvent extraction and to study the rate of synthesis of these fractions under deficient and normal conditions. The technique of growing the plants was the same as in the previous experiments. Ten plants were selected from each treatment for the tracer work. These were placed in two-quart Mason jars filled with nutrient solution containing radiophosphorus. At intervals, plants were taken from each group and subjected to the following treatment: the entire plant was dried at 80° C. overnight, then ground to a powder in a Wiley mill. The material was thoroughly mixed, weighed, and a sample weighed out to fill a 22 × 80-mm. Soxhlet extraction thimble. This sample was extracted continuously in a Soxhlet apparatus for 24 hours with petroleum ether (b.p. 30° to 60° C.). The extract was made up to 100 ml. in the extraction flask (previously calibrated), an aliquot was measured into a crucible, dried, and counted. After completion of the extraction with petroleum ether, the samples were dried and extracted in the same apparatus for 24 hours with 95 per cent. ethanol. This extract was treated in the same manner as the ether extract. The samples were then dried throughly in an oven at 80° C. and transferred to large fluted filters. They were then extracted by repeated washings with two per cent. hydrochloric acid, catching the filtrate in a 500-ml. volumetric flask and continuing the extraction procedure until the volume of the filtrate reached 500 ml. This filtrate was thoroughly mixed and an aliquot measured into a crucible, dried, and the radioactivity determined. The residue on the filter paper was then transferred to a 250-ml. beaker and dried at 80° C. It was then weighed, and a sample weighed into a crucible and ashed before counting.

Results

Experiment 1

The forty plants used in this experiment were transplanted to glazed ½-gallon crocks on January 31, 1942. The pots were flushed daily with fresh nutrient solution which was maintained at pH 4.8 to 5.0 in all cases. By February 28 the plants on the nitrate treatment were 8 to 10 inches in height, and were thicker stemmed and larger in every respect than the plants receiving urea which were 1 to 2 inches shorter. The color of the plants on both treatments was about the same. At this time the phosphorus was omitted from the nutrient solution supplied to the plants on the no-phosphorus treatments. The plants were continued on these treatments until definite signs of phosphorus deficiency had appeared in the plants which received no phos-

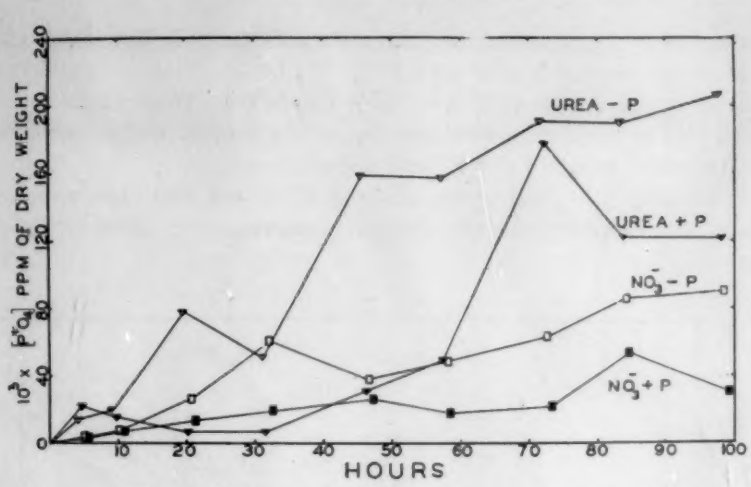

FIG. 1. Absorption of $P^*O_4^{\equiv}$ by tomato leaves. All nutrient solutions were maintained at pH 4.8 to 5.0.

phorus. About 3 weeks after the removal of phosphorus the appearance of the four groups of plants was as follows:

Group 1. Nitrate plus phosphorus ($+NO_3^- + P$). Average height 28 inches, light green in color, luxuriant growth, thick stems.

Group 2. Nitrate minus phosphorus ($+NO_3^- - P$). Average height 26 inches, dark green in color, pronounced purpling of midribs and underside of leaves, especially in the upper regions of the plants. In general, the plants were thinner stemmed than those in group 1. Small dark spots were visible on the stems. In many instances the younger leaves had a tendency to curl under.

Group 3. Urea plus phosphorus ($+\text{Urea} + P$). Average height 18

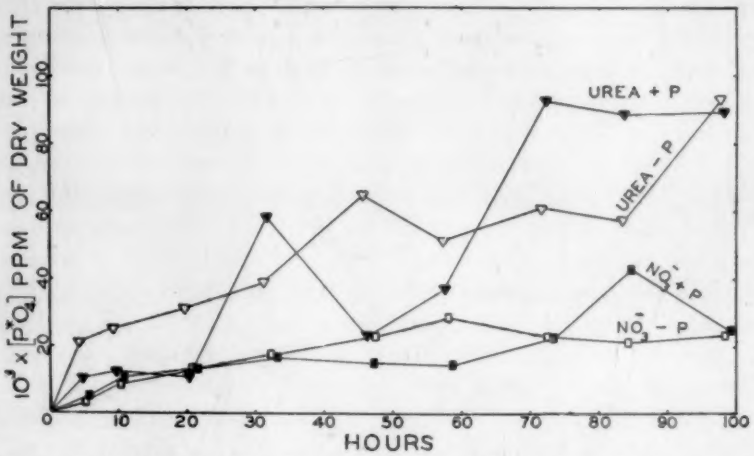

FIG. 2. Absorption of $P^*O_4^{\equiv}$ by tomato stems. All nutrient solutions were maintained at pH 4.8 to 5.0.

inches, average green color. Plants were well formed, but much smaller than those on the nitrate treatment.

Group 4. Urea minus phosphorus (+ Urea – P). These plants appeared to be slightly more spindly than those in group 3, but the differences in color, noticed between groups 1 and 2, were not observed.

At this time the plants were about 8 weeks old and they were transferred to water cultures for the absorption experiments. Results are given in figures 1, 2, and 3.

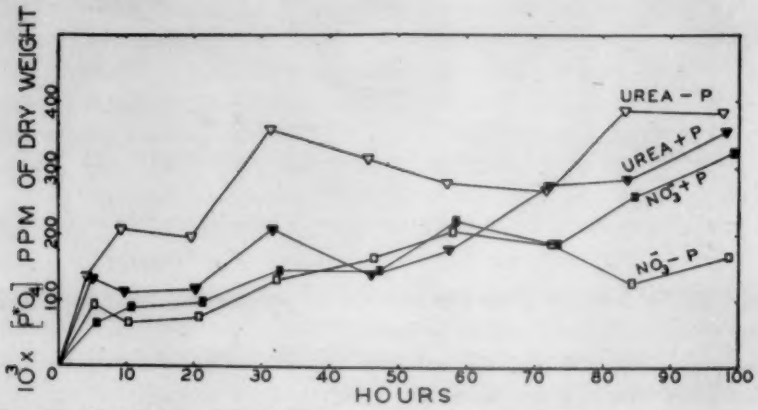

FIG. 3. Absorption of $P^*O_4^{\equiv}$ by tomato roots. All nutrient solutions were maintained at pH 4.8 to 5.0.

EXPERIMENT 2

In every case, the tomato plants made poor growth when supplied at pH 4.8 with urea. Since it has been shown (**5, 6, 11**) that ammoniacal nitrogen is more rapidly absorbed by the tomato at higher pH levels and that tomato plants make better growth on ammonia nitrogen in a more alkaline medium (**4**), it was decided that perhaps a more nearly normal set-up for studying the phosphorus absorption rate by plants receiving nitrate nitrogen and plants receiving urea nitrogen would be to grow the plants receiving urea in solution cultures maintained at pH 6.8 to 7.0. The procedure followed was the same as in experiment 1, except that from the time of transplanting (March 7, 1942) the nutrient solution added to the urea groups was kept at pH 6.8 to 7.0 by the addition of normal potassium hydroxide solution.

From the start it was evident that the plants furnished urea were making much better growth at pH 6.8 to 7.0 than they had previously made at pH 4.8 to 5.0. This was corroborated by the fresh and dry weights of the plant tissues (**3**). At no time before phosphorus was removed from groups 2 and 4 was there any noticeable difference in appearance between the nitrate and urea series of plants. Both grew vigorously, appeared light green and healthy. After the removal of phosphorus from these two groups, deficiency symptoms appeared first in group 2 (plants receiving nitrate but no phosphorus) and were followed by group 4 (plants receiving urea but no phosphorus) in about 5 to 6 days. This delay in the appearance of deficiency

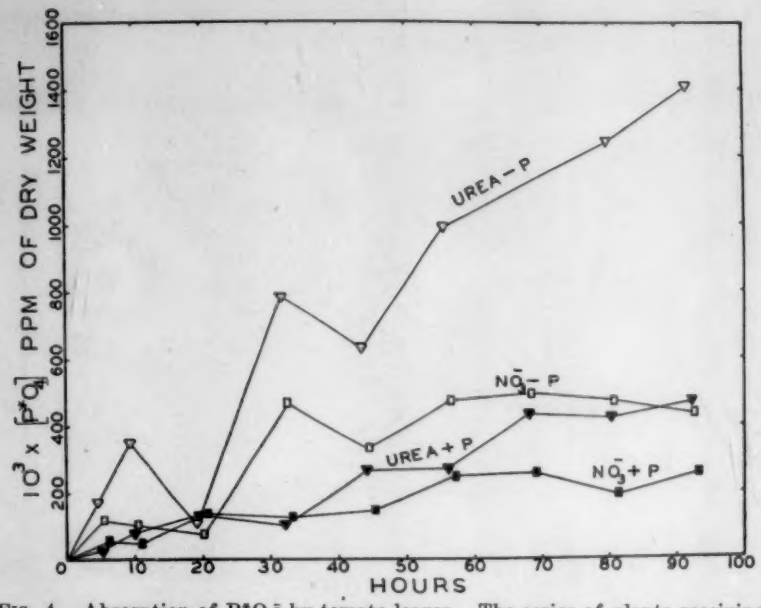

FIG. 4. Absorption of $P^*O_4^{\equiv}$ by tomato leaves. The series of plants receiving urea were grown at pH 6.8 to 7.0; the series receiving nitrate at pH 4.8 to 5.0.

symptoms was not as noticeable as it had been at the lower pH, however. Results of experiment 2 are given in figures 4, 5, and 6.

EXPERIMENT 3

Plants used in this experiment were about 10 weeks old on April 20, 1942, and consisted of 2 lots; one grown in a medium supplying nitrate and phosphorus (group 1), and the other in one supplying nitrate but no phosphorus

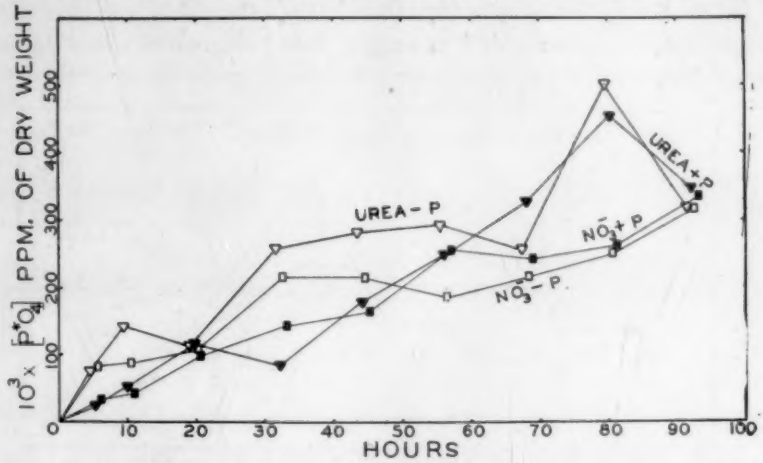

FIG. 5. Absorption of $P^*O_4^{\equiv}$ by tomato stems. The series of plants receiving urea were grown at pH 6.8 to 7.0; the series receiving nitrate at pH 4.8 to 5.0.

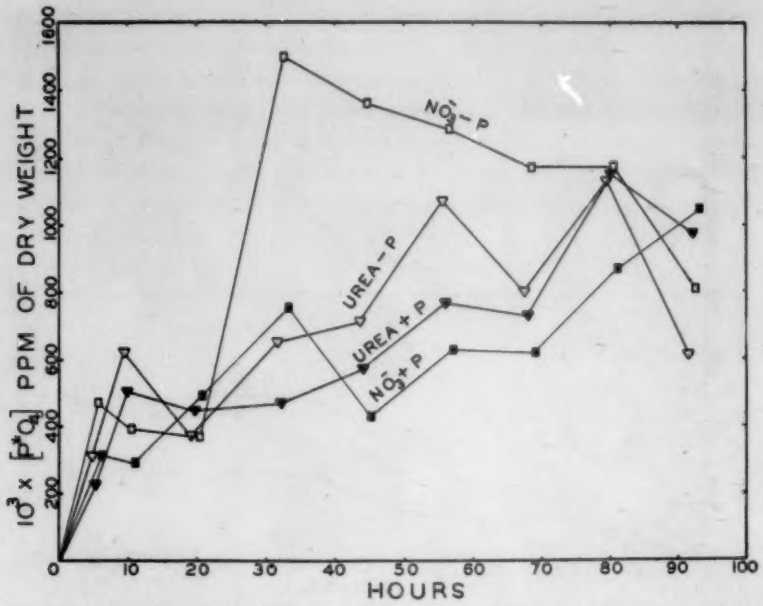

FIG. 6. Absorption of $P^*O_4^{\equiv}$ by tomato roots. The series of plants receiving urea were grown at pH 6.8 to 7.0; the series receiving nitrate at pH 4.8 to 5.0.

(group 2). The plants in group 2 were suffering from phosphorus starvation to about the same degree as the plants in the previous experiments. The data from this experiment are presented in figures 7, 8, 9, and 10. It will be observed that the data are expressed as percentage p.p.m. $P^*O_4^{\equiv}$, dry weight basis. It was thought that this means of expression would indicate more clearly the relationship of each fraction to the others.

Discussion

From the data presented, it is evident that phosphorus was absorbed at a more rapid rate by the phosphorus deficient plants than by the normal

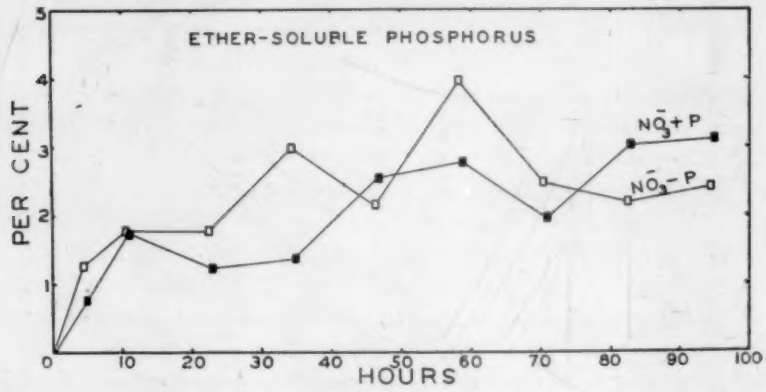

FIG. 7. Distribution of newly absorbed phosphorus in phosphorus deficient plants, and in plants receiving adequate phosphorus.

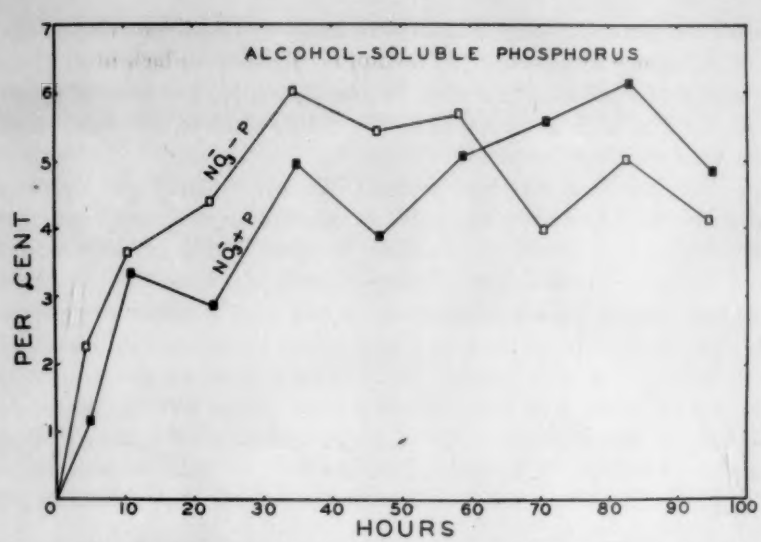

FIG. 8. Distribution of newly absorbed phosphorus in phosphorus-deficient plants, and in plants receiving adequate phosphorus.

plants (those receiving adequate phosphorus); also that the plants furnished urea absorbed phosphorus much more rapidly than those furnished nitrate. The results of experiment 2 illustrate that an increase in alkalinity of the nutrient solution furnishing urea to the plants resulted in increased growth and vigor of these plants but had no effect on the increased phosphorus absorption of these plants over the plants receiving nitrate. These findings merely show that a deficiency in the plant increases the rate of absorption and that the form in which nitrogen is available to the plant apparently influences the rate at which phosphorus is taken up by the plant. Preliminary work and analyses described in another paper (3) show that the amount of phosphorus per unit weight of plant is also influenced by the

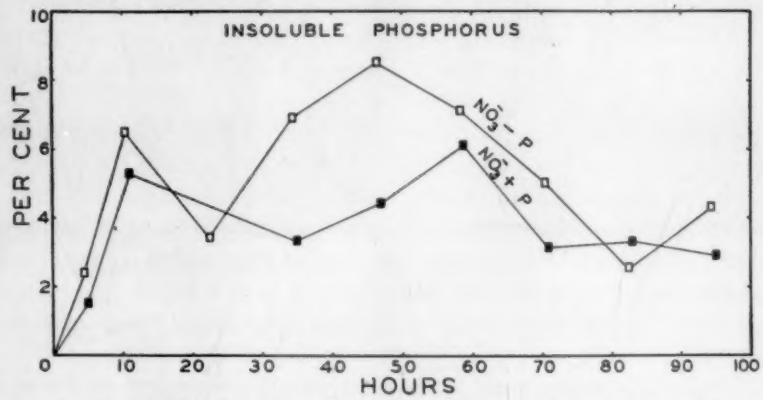

FIG. 9. Distribution of newly absorbed phosphorus in phosphorus-deficient plants, and in plants receiving adequate phosphorus.

form of nitrogen supplied. It is evident therefore that the delayed appearance of deficiency symptoms due to lack of phosphorus in plants receiving urea as a source of nitrogen may be due to the higher reserve supply of phosphorus available to these plants (3) rather than to a disruption of the nitrate reduction mechanism.

An experiment of this type is quite difficult to carry out by ordinary chemical methods because of the small differences in phosphorus content that would have to be detected chemically. Because of the extreme sensitivity and the nature of the radioactive tracer technique it is possible to differentiate between newly absorbed phosphorus and that already present in the plant. In explanation of the fact that the data are plotted in broken rather than smooth curves, it is pointed out that each point on the graphs represents a single plant, and that individual variations make it impossible to obtain smooth curves in this type of study unless large numbers of plants are used. It is thought, however, that the data do indicate general trends

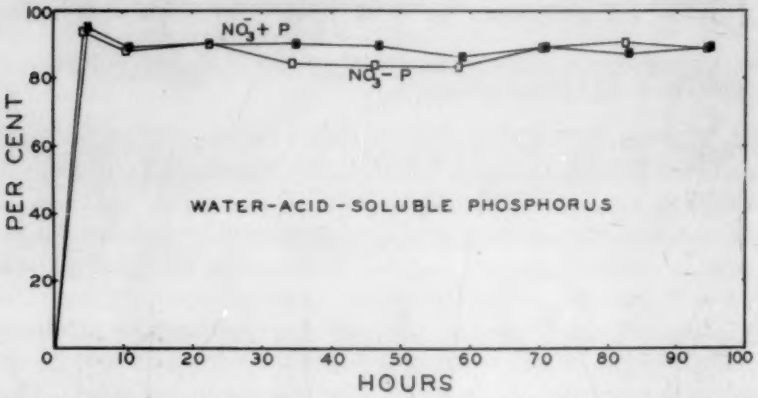

FIG. 10. Distribution of newly absorbed phosphorus in phosphorus-deficient plants, and in plants receiving adequate phosphorus.

in the absorption rate and that the differences between treatments are significant. The differences between phosphorus-deficient and normal plants are small when the stems are used as a source of material, as is to be expected.

The data from experiment 3 are of interest in pointing out that when phosphorus is supplied to plants growing in a medium lacking phosphorus the phosphorus absorbed by the plant is distributed among the phosphorus-containing compounds of the plant in about the same proportion as it is in plants growing in a solution containing an adequate amount of phosphorus. As is to be expected, however, the percentage of ether soluble, alcohol soluble, and residue phosphorus in the deficient plants is on a higher level than that in the normal plants, while the percentage water soluble phosphorus is on a lower level.

Although this experiment gives no clear-cut separation of the various phosphorus-containing compounds in the plant, it certainly separates them into groups, the composition of which may be fairly accurately estimated.

The petroleum ether fraction consists predominantly of phospholipides. The alcohol extract consists of low molecular weight organic phosphorus compounds such as the phosphoric acid esters and other compounds which function in the intermediary metabolism of the plant foods, and perhaps a small amount of the water soluble phosphorus. The water-acid fraction consists almost entirely of the inorganic phosphorus and part of the phytin. Recent evidence (**2**) indicates that in the presence of large amounts of iron the major part of the phytin may remain in the tissues as an insoluble iron complex. The residue fraction, then, represents a part of the phytin, and other insoluble phosphorus compounds such as the nucleoproteins.

Summary

1. Tomato plants grown in a medium supplying urea as a source of nitrogen did not develop deficiency symptoms due to lack of phosphorus as soon as did plants which received nitrate nitrogen. This was true of plants grown at both pH 4.8 to 5.0 and pH 6.8 to 7.0, although the plants which received urea at the higher pH did not show as marked a delay in the appearance of the symptoms as did those at pH 4.8 to 5.0. Plants furnished urea at pH 6.8 to 7.0 made much better growth than at pH 4.8 to 5.0.

2. Radiophosphorus, as disodium phosphate, was employed to measure the uptake rate of phosphorus by normal and phosphorus deficient tomato plants grown in two separate media; one supplying nitrate, the other urea, as sources of nitrogen.

3. Tomato plants which received urea as a source of nitrogen absorbed phosphorus at a greater rate than did plants which received nitrate nitrogen. Along with the higher phosphorus content of the plants which received urea this was interpreted as evidence that the delay in the appearance of deficiency symptoms in these plants was due to their increased phosphorus reserve rather than to a partial breakdown in their nitrate reduction mechanism.

4. Phosphorus deficient plants were shown to absorb phosphorus at a greater rate than normal plants (those receiving adequate phosphorus) grown in the same solution.

5. Measurement of the distribution of newly absorbed phosphorus by deficient and normal plants indicated that the various phosphorus compounds of the plant were synthesized in about the same proportions by the phosphorus-deficient plant as by the normal plant but at a greater rate.

The radiophosphorus was generously supplied by Dr. J. H. Lawrence of the Crocker Radiation Laboratory, University of California, Berkeley, California.

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SUGAR MOVEMENT TO ROOTS, MINERAL UPTAKE, AND THE GROWTH CYCLE OF THE COTTON PLANT[1]

FRANK M. EATON AND HOWARD E. JOHAM

Comparative data are reported in this paper on the sugar concentrations in the fibrous roots (and other parts) of defruited and control cotton plants and the uptake by these plants of bromine, nitrogen, and potassium. The measurements were made 22 days after defruiting (21 days after the addition of bromine to the nutrient solution). The data are discussed in terms of the relation of sugar concentrations in fibrous roots to mineral uptake and the relation of mineral uptake to the growth cycle of the cotton plant.

The uptake of soil nutrients by the cotton plant, as shown by WHITE (**27**), CROWTHER (**8, 9**), DASTUR (**10**), OLSON and BLEDSOE (**23**), and others, proceeds at an increasing rate through the period of early growth and flowering but subsequently, as the number of bolls carried by the plants increases, the rate of uptake declines and actual losses of mineral elements from the above-ground portions of the plants may occur. It was suggested a number of years ago (**8**) that the observed reduction in nitrogen uptake during the fruiting period of cotton might be due to depressed root growth. The dominance of fruiting activity over vegetative development and root growth has been well established in cotton (**12, 13, 19, 20**) and it seems certain that the extension of roots into new soil zones and the development of new root surfaces would be important factors in solute uptake. The foregoing explanation, however, insufficiently accounts for all of the known facts. If, on the other hand, fruiting substantially reduces the concentration of sugars in the fibrous roots, the observed reduction in mineral uptake with fruiting can be more fully comprehended.

The necessity for an expenditure of energy for the accumulation of solutes against concentration differentials has been outlined by HOAGLAND (**17**) and more recent work with barley seedlings (**5**) adds assurance that sugars situated in the roots provide the primary respiratory fuel for mineral uptake. It has been shown (**18, 20**) that the developing cotton boll tends to have first call on labile carbohydrates. Irrespective of probabilities, however, it has not been established that the sugar concentrations in fibrous roots vary enough with fruiting to constitute a critical intermediate link between fruiting and the rate of mineral uptake. The responses of soybeans (**3**) and of tomatoes (**2**) to defloration indicate that fruiting may alter the carbohydrate relations in the above-ground portions of plants without affecting materially the rate of mineral uptake. Two additional papers (**24, 25**) also pertain to the foregoing considerations.

In soybeans, AUSTIN (**3**) found the accumulation of some elements was depressed by defloration; his data show, however, that defloration had little

[1] Published with the approval of the Director as Technical Paper no. 841 of the Texas Agricultural Experiment Station.

effect on sucrose concentrations in stems and leaves and it substantially decreased reducing sugars and increased polysaccharides. In the tomato, HESTER (**15**) found no drop in mineral uptake with fruiting, and ARNON and HOAGLAND (**2**) concluded that deflorating tomato plants did not result in marked increases in potassium, calcium, magnesium, or phosphorus in above-ground vegetative organs. There were, however, higher concentrations of organic nitrogen and sugars in the stems and leaves.

Remarkable parallelisms are brought out by MILLER's data (**22**) between the levels of sugars in stems and leaves and the movement of minerals into the tops of wheat plants with heading. Relationships that seem to be quite direct have been reported likewise by COCHRAN (**7**) between depressed mineral accumulation and fruiting in pimiento and by CAROLUS (**6**) with the formation of tubers by potatoes. A decline in mineral uptake with advance in age has been found in tobacco by GRIZZARD, DAVIES, and KANGAS (**14**). ANDERSON, SWANBACK, and STREET (**1**) have assigned the loss of nitrogen and potassium from the leaves of topped tobacco plants to translocation to the suckers. In the majority of the foregoing papers causal mechanisms are not discussed and, except as mentioned, sugar determinations were not involved in the experimental work. MILLER (**21**) has made reference to relations between fruiting and the mineral uptake by additional plants.

Methods

Stoneville 2–B upland cotton was planted in the greenhouse in 3-gallon stone jars filled with river sand on March 18. Flowering started on May 10 at which time the plants were taken outdoors. They were supplied with a nutrient solution containing millimolar concentrations of salts as follows: 2 calcium nitrate, 2 magnesium sulphate, 3 potassium chloride, and 0.3 potassium dihydrogen phosphate; in addition 5 p.p.m. of boron, 0.5 p.p.m. of manganese, and 0.05 p.p.m. of zinc; iron was supplied as magnetite mixed with the sand. The use of the above solution was continued until June 1 except that on May 1, the 3 millimoles of potassium chloride were replaced with a like amount of potassium sulphate. Throughout the experiment the nutrient solution was supplied in sufficient excess to produce substantial drainage.

Sugars, after extracting the dried tissues with hot 80 per cent. alcohol, were determined by the WILDMAN and HANSEN (**28**) procedure except that in titration potassium permanganate oxidizing solution was employed with ortho-phenanthrolic indicator; starch was hydrolyzed in the residue from the sugar extraction with undiluted plant diastaste (supplied by courtesy of Parke, Davis and Company); bromine was determined according to Hibbard (**16**), and nitrogen (including nitrate) by A. O. A. C. official methods (**4**). Potassium was determined by the chloroplatinate method outlined by ST. JOHN and MIDGLEY (**26**).

Calendar

MAY 28

Eighteen jars were numbered and the heights of the plants measured. The plants were then divided into six groups of 3 plants each in such manner that the average height of the plants in each group would be approximately equal. Three groups were used for the defruited and 3 groups for the control treatment. The individual pots remained in their initial locations and their positions were therefore randomized as to treatment.

MAY 31

All bolls were removed from 3 sets of plants (defruited) and the large squares (floral buds) were subsequently removed before they flowered.

JUNE 1

Potassium bromide was substituted for potassium sulphate. The composition of the culture solution was otherwise altered at this time to give concentrations in millimoles per liter as follows: 8 $Ca(NO_3)_2$, 8 $MgSO_4$, 12 KBr, and 1.2 KH_2PO_4.

JUNE 22

Collection of samples. Material from the 3 plants of each group was combined. The order of sample collection was as follows:

12:30 P.M. C.S.T. Collected 4 sound main-stalk leaf blades (from the 11th to the 14th or 15th nodes) from each plant. These were weighed green and then dried for 2 hours at 80° C. under forced draft; drying was completed at 70° C.

1 to 2 P.M. Cut off the tops of all plants after removing the bolls from the control plants. These two fractions (bolls and combined leaves and stems) were weighed fresh and dried on the following day at 70° C.

2 to 4 P.M. The sand was washed from the pots and the roots rewashed in the laboratory under a jet. The fibrous and tap roots were separated and blotted, weighed fresh, and dried immediately in the same manner as the leaves.

Results

GROWTH OF CONTROL AND DEFRUITED PLANTS

During the 22 days following boll removal the defruited plants elongated at their terminal buds about twice as rapidly (table I) as the control plants. By the end of this period the fresh weight of fibrous roots of the defruited plants was double that of the control plants; lesser relative gains were found in the weights of the tap roots (included large laterals) and in the weights of the combined leaves and stems. The weights of tops plus roots (exclusive of bolls) at the end of the three-week period were, respectively, 503 and 799 grams for the control and defruited treatments; the weights of the bolls initially removed from the "defruited plants" were not recorded but had these weights been added to the final weights the results would indicate more

TABLE I

GROWTH AND FINAL FRESH WEIGHTS OF CONTROL AND DEFRUITED COTTON PLANTS. EACH GROUP VALUE IS THE MEAN OF 3 PLANTS

TREATMENT	GROUP	HEIGHT				FINAL FRESH WEIGHT				
		INITIAL	INCREASE BY WEEKS			BOLLS	ROOTS		LEAVES AND STEMS	TOTAL
			1ST	2ND	3RD		FIBROUS	TAP		
		cm.	*cm.*	*cm.*	*cm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control	A	55.0	4.0	5.0	6.0	222	157	16.0	297	693
	B	56.0	6.0	6.0	5.0	195	142	17.0	299	655
	C	59.0	8.0	7.0	6.0	205	194	23.0	360	783
	Mean	56.7	6.0	6.0	5.7	207	164	18.7	319	710
Defruited	A	57.0	12.0	13.0	13.0	.....	324	24.0	417	766
	B	58.0	11.0	13.0	12.0	.....	363	30.0	492	885
	C	57.0	9.0	10.0	13.0	.....	304	29.0	414	747
	Mean	57.3	10.7	12.0	12.7	.....	330	27.7	441	799

definitely that the total dry matter accumulation by the defruited plants was greater during the experimental period than by the fruited plants (compare **12, 19**).

BROMINE ACCUMULATION PER PLANT

The defruited plants accumulated almost twice as much bromine in the 3-week period as did the control plants (table II). The fraction of the total bromine found in the bolls was comparatively small; in terms of fresh weight the concentration of bromine in the bolls was approximately one third as great as in the corresponding main-stalk leaves.

CONCENTRATIONS OF BROMINE AND SUGARS IN FIBROUS ROOTS AND OTHER TISSUES

The sugar concentrations in the fibrous roots (table III) were increased

TABLE II

BROMINE ACCUMULATION IN ENTIRE PLANTS

TREATMENT	GROUP	MILLIGRAMS BROMINE PER PLANT				
		BOLLS	FIBROUS ROOTS	TAP ROOTS	LEAVES AND STEMS	ENTIRE PLANTS
		mg.	*mg.*	*mg.*	*mg.*	*mg.*
Control	A	68.3	231	18.7	685	1003
	B	77.3	347	27.4	749	1200
	C	63.6	367	45.4	1154	1630
	Mean	69.7	315	30.5	863	1278
Defruited	A	.....	1003	40.0	1247	2290
	B	.....	983	51.3	1615	2649
	C	.....	791	49.0	1162	2003
	Mean	.....	926	46.8	1341	2314

by defruiting approximately three fold but this difference did not meet the requirements of the 0.05 level of significance; starch concentrations found in the fibrous roots of the defruited plants were significantly higher than those found in the control plants. Corresponding to these differences in carbohydrate concentrations there was an increase of approximately sixty per cent. in the concentration of bromine in the fibrous roots of the defruited versus the control plants and this difference was highly significant. The concentrations of bromine found in the main-stalk leaves are correlated with the concentrations of sugar in the fibrous roots but they are not correlated with the concentrations of sugar in the leaves.

TABLE III

CONCENTRATIONS OF BROMINE AND CARBOHYDRATE IN FIBROUS ROOTS, TAP ROOTS, AND LEAVES OF CONTROL AND DEFRUITED COTTON PLANTS AND RESULTS OF ANALYSIS OF VARIANCE

Treatment	Group	Milligrams per gram fresh weight								
		Fibrous roots			Tap roots			Main-stalk leaves		
		Bromine	Total sugars	Starch	Bromine	Total sugars	Starch	Bromine	Total sugars	Starch
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Control	A	1.60	0.078	1.95	1.17	5.11	13.7	0.95	3.11	13.6
	B	2.27	0.156	1.95	1.56	5.42	13.2	0.99	2.99	13.6
	C	1.65	0.073	1.94	2.01	4.86	13.7	1.70	2.61	12.5
	Mean	1.84	0.102	1.95	1.58	5.13	13.5	1.21	2.90	13.2
Defruited	A	3.03	0.245	2.12	1.58	13.10	33.7	2.10	3.53	12.7
	B	2.72	0.205	2.13	1.63	14.57	31.3	2.45	1.91	10.4
	C	3.01	0.451	2.26	1.89	14.66	25.5	1.84	3.33	12.8
	Mean	2.92	0.300	2.17	1.70	14.11	30.2	2.13	2.92	12.1
Mean difference		1.08	0.198	0.22	0.12	8.98	16.7	0.92	0.02	1.1
Required m.d. for sig. to 0.05		0.64	0.224	0.11	0.73	1.50	6.8	0.82	0.59	2.4

There was no difference in the concentrations of sugar or starch in the main-stalk leaves under the two treatments of this experiment. Samples collected earlier in the day in other experiments have shown higher carbohydrate concentrations in leaves from defruited plants than in those from fruited plants. MASKELL and MASON (**18**) likewise have found higher concentrations of both nitrogen and sucrose in the leaves, bark, and wood of defruited than of control cotton plants (average of 2 morning and 1 afternoon samples). In the control plants the sugars from the leaves presumably moved principally into the bolls, whereas in the defruited plants a part of the sugar that would otherwise have moved into the bolls was utilized in a greater growth of leaves and framework, but a relatively greater proportion of it was utilized in the extensive development of the fibrous roots.

Accumulation of nitrogen and potassium

Bromine was introduced in the culture solution as the test element on the day following defruiting whereas the other mineral elements of the nutrient solution were all present from the start. Analyses for the concentrations of total nitrogen and of potassium in the fibrous roots and in the main-stalk leaves were made out of general interest and these results, together with a recapitulation of the sugar and bromine data, are reported in table IV.

TABLE IV

Concentrations of sugars, bromine, total nitrogen, and potassium in fibrous roots and main-stalk leaves

Treatment	Group	Milligrams per gram fresh weight							
		Fibrous roots				Main-stalk leaves			
		Sugars	Bromine	Nitrogen	Potassium	Sugars	Bromine	Nitrogen	Potassium
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Control	A	0.078	1.60	1.26	0.57	3.11	0.95	5.87	2.36
	B	0.156	2.27	1.49	0.69	2.99	0.99	6.02	1.93
	C	0.073	1.65	1.63	0.64	2.61	1.70	6.40	2.25
	Mean	0.102	1.84	1.46	0.63	2.90	1.21	6.10	2.18
Defruited	A	0.245	3.03	1.75	0.92	3.53	2.10	5.73	1.80
	B	0.205	2.72	1.69	0.91	1.91	2.45	5.73	2.84
	C	0.451	3.01	1.94	0.90	3.33	1.84	5.78	2.73
	Mean	0.300	2.92	1.79	0.91	2.92	2.13	5.75	2.46
Mean difference		0.198	1.08	0.33	0.28	0.027	0.916	0.35	0.28
Required m.d. for sig. to 0.05		0.224	0.64	0.38	0.19	0.574	0.817	0.44	0.98

Higher concentrations of nitrogen and potassium, as well as of bromine, were found in the fibrous roots of the defruited plants. The increase in potassium was substantial but the difference in nitrogen lacked statistical significance. In the main-stalk leaves significant differences were found only in the instance of the bromine concentrations.

Discussion

Although there was no overlapping between treatments in the concentrations of sugar, bromine, nitrogen, or potassium in the fibrous roots, there was a marked variability within the treatments. If the observed increases in accumulation as reflected by the concentration of bromine, nitrogen, and potassium in the fibrous roots were actually produced by reason of the higher sugar concentrations, then the differences in accumulation should be directly correlated with the differences in sugar concentrations—irrespective of treatment. This aspect of the problem has been examined by calculating the coefficients of correlation between the concentrations of sugar and the concentrations of bromine, nitrogen, and potassium in the fibrous roots.

The values are as follows (a value of 0.811 is required for significance at 0.05): bromine, 0.844; nitrogen, 0.828; potassium, 0.781.

The fact that these coefficients of correlation are all positive and all high, with two of the three significant, provides additional evidence that the relationship between the level of sugars and the level of solute accumulation in the roots is a causal one. Significant correlations between sugars in the roots and minerals in the leaves were not found and in this experiment they were not necessarily to have been expected, since in terms of concentrations as differing from total accumulation the dilution effects associated with the more rapid growth of the defruited plants would parallel in direction the exhaustion effects resulting from the reduced uptake by the control plants.

It seems probable that above some relatively low concentration of labile carbohydrate in a root further increases would have little effect upon the rate of metabolic activity and in turn upon the rate of solute accumulation. It is worthy of note in this connection that there were like concentrations of bromine in the fibrous roots of the defruited A and C groups (table III) notwithstanding a nearly doubled concentration of sugar in the latter. Likewise there was no indication of a relationship between the sugar concentrations in the leaves, where sugars were much higher than in the fibrous roots, and the concentration of bromine in the leaves; and yet the uptake of bromine by leaf cells from the xylem vessels, or the retention of bromine by them, requires the expenditure of metabolic energy if the concentrations of labile ions in the leaf cells are greater than in the vessels.

It is useful to speculate on the indications afforded by the data with respect to the sugar concentrations in the active terminal regions of the fibrous roots under the two treatments. The sugar concentrations found in the masses of fibrous roots are probably excellent indices to the concentrations in their terminal regions but it is not to be assumed that the one is a direct measure of the other. The sugars in the tap roots (fraction included large laterals) were 50 times more concentrated than in the masses of fibrous roots under both treatments and yet it is reasonable to believe that at the points of attachment the concentrations in the fibrous roots tended to approach the concentrations in the tap roots. At the terminal ends of the fibrous roots the concentrations would of necessity, therefore, have been very much lower than the concentrations found for the masses as a whole; the values ranging from 0.0073 to 0.0451 per cent. (table IV) found for the concentrations of sugars in the masses of fibrous roots would suggest that the concentrations existing in the terminal regions of these roots must have been extremely low.

Observations that have been made on the growth reactions of the cotton plant are such as to indicate that the extent to which vegetative development is slowed down with the onset of fruiting is related not only to the degree of fruitfulness but also to the concentration of nutrients in the substrate. It seems reasonable that an interrelation between the two factors should exist since on theoretical grounds a greater expenditure of metabolic energy would

be required for the uptake of solutes from dilute than from concentrated substrates. The writers cannot recall ever having seen dormant terminal buds on cotton plants growing in sand cultures abundantly supplied with nutrients. Dormant buds are readily obtained, however, when the nitrate supply is sufficiently reduced. Plants growing on low-nitrogen soils in containers have been stimulated into improved growth and color either by the addition of nitrogen or by the removal of all previously set bolls; the two treatments appear to have similar effects, which is reasonable on the basis of metabolic consideration, since with an increased movement of sugar to the roots an increased uptake from the dilute substrate should follow. Renewed vegetative activity is almost always observed following the maturation of the first crop of bolls in the late summer.

Under field conditions the extension of roots into new soil zones makes available additional supplies of nutrients but in sand cultures this consequence of root growth would not exist. It is entirely possible, nonetheless, that with a greater number of roots the uptake of mineral elements might increase. Although neither of the foregoing considerations would seem to adequately account for the negligible, or even negative, uptake of soil nutrients by heavily fruited plants with depressed root development, the fact remains that in the present experiment the defruited plants developed greater root systems relative to the weight of their tops than did the control plants and that the defruited plants accumulated a greater weight of bromine. It seems reasonable to assign some part of the extra bromine uptake by the defruited plants to their extra roots but such an assignment in no way accounts for the higher *percentages* of bromine in the fibrous roots of the defruited plants; the higher percentages of bromine are most logically explained on the basis of the higher sugar concentrations. The concentrations of nutrients were made relatively high following defruiting in this experiment and the possibility was thereby avoided that lack of availability of any nutrient might have limited its uptake. On the basis of energy relationships it would seem that more outstanding differences would have resulted had the cultures been supplied with frequently replaced but very dilute solutions.

Although existing information leaves uncertain the manner of operation of many physiological relationships involved in the growth and fruiting of cotton, the finding of a direct relation between carbohydrate movement into the fibrous roots and mineral uptake makes possible a further projection of the cause and effect factors involved in the growth cycle. In undertaking to fit the foregoing findings into the existing information on this subject an omission is purposely made of all reference to the effects of drought. This omission may not be justified, particularly as it is known that there are substantial increases in the concentrations of carbohydrates in the cotton plant when the water supply is insufficient. The effect of drought on mineral uptake by cotton, however, has not been studied and many other physiological relationships are undoubtedly altered by high water tensions.

Limiting the present discussion to plants amply supplied with moisture, the physiological sequence of events, in accord with present information, would appear to be as follows: During the preflower and early flowering period photosynthetic products are rapidly converted into above-ground vegetative structures and root growth and there is an associated heavy uptake of soil nutrients. With flowering and the continued setting of bolls more and more of the carbohydrates and of the current and previously accumulated nitrogen (**18**) are moved to the bolls. The reduction in the movement of carbohydrates to the roots reduces mineral uptake and this reduction would in turn be expected to restrict growth. Accompanying the curtailed vegetative expansion, the effectiveness of the older leaves declines and the former upward trend in photosynthetic output (**8**) is flattened or reversed. By this stage many of the young bolls are shedding. The rates of shedding, flowering, and growth fluctuate some with changes in environmental conditions but the fluctuations are secondary to the broad trend of the cycle. Should the reciprocally depressing effects of reduced carbohydrates and reduced mineral uptake become sufficiently pronounced, the terminal bud of the plant becomes dormant and leaf and flower development ceases. With the maturation of the crop of bolls the cotton plant either dies (exceptional), as do annuals, or it resumes growth as do perennials—possibly depending upon the extent of its depletion.

In accord with the early conclusions of EWING (**13**) and MASON (**19**) there seems to be little room for doubt that much of the shedding of cotton bolls (exclusive of that caused by bud and boll insects) reflects a reduction in the number of bolls carried by the cotton plant to levels compatible with the plant's current ability to maintain boll growth. Daily graphs of shedding rates are typically characterized by major trends that are broken by successions of upward and downward variations. These marked variations in shedding suggest that the stimulus, whatever its nature, that gives rise to abscission may often cause the loss of more bolls than is necessary to reduce the boll load to a level compatible with the carrying capacity of the plant. Reductions below carrying capacity would account for the marked increases in flowering, boll setting, and growth that so often follow heavy shedding or that always follow the artificial removal of bolls. Likewise, it seems as logical to look for high shedding following a return to customary conditions after especially favorable weather as after unfavorable weather following customary conditions. Under environmental conditions sufficiently uniform as to avoid the waves of shedding and the subsequent momentary increases in growth and flowering rates, it seems probable that the interrelations between fruiting, mineral uptake, and growth would be even more clearly defined than they are under varying climatic conditions.

DASTUR and AHAD (**10**) working in the Punjab found maximum mineral uptake during the early flowering stages of both American and *desi* cottons. The *desi* cotton plants were more fruitful than the American and the authors noted that "As a rule the leaves of the *desi* plants get depleted of their

minerals more than the leaves of the American." The two varieties were alike as regards leaf nitrogen. Other than for the work just cited there is little basis for a conclusion on whether material differences exist in the rates of mineral uptake by highly determinate strains of cotton and strains that shed a greater proportion of their bolls and for a longer time continue to make vegetative growth. In those regions where diseases, insects, or climatic conditions are incompatible with late cotton, the early determinate strains are most popular but elsewhere the less determinate strains are usually found to be more productive. Under the latter conditions the artificial removal of all early flowers has resulted in higher yields (**12**); but in Mississippi (**15**), the removal of all squares (floral buds) during the first one to six weeks of the flowering period was without consistent effect on final yields and the larger plants were regarded as undesirable. The fact that yields were not depressed by square removal over long periods during the forepart of the flowering period strongly suggests that occasional bursts of shedding may be of minor significance.

Summary

The uptake of mineral elements has been shown by others to proceed rapidly in the cotton plant during its early growth period but to decline or become negligible with the setting of a large number of bolls. In this paper the effect of fruiting upon the sugar concentrations in the fibrous roots and in turn upon bromine uptake was studied by comparing fruited and defruited plants grown in sand cultures.

By the end of a period of three weeks, following the addition of bromine to the nutrient solution and the removal of flower buds and bolls, the concentration of sugar in the fibrous roots had tripled and the concentration of bromine had increased 60 per cent. over the control plants. The weight of bromine accumulated by entire defruited plants was double that found in the control plants. The fibrous roots of the defruited plants had a slightly higher concentration of nitrogen and a significantly higher concentration of potassium at the end of the experiment.

It appears that much of the decline in mineral uptake with heavy fruiting can be attributed to the reduced movement of carbohydrate to the roots.

The reduced vegetative expansion of the cotton plant that occurs during the period of heavy fruiting is undoubtedly due both to the utilization of carbohydrates by developing bolls and to the reduced mineral uptake. When these reciprocally depressing effects are sufficiently pronounced, vegetative growth becomes negligible. With the maturation of a crop of bolls the cotton plant either dies (exceptional) as do annuals, or it resumes growth as do perennials—possibly depending on the extent of its depletion.

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RELATION OF THE PRODUCTION OF AN ACTIVE EMANATION TO RESPIRATION IN THE AVOCADO FRUIT

HARLAN K. PRATT AND J. B. BIALE

(WITH SEVEN FIGURES)

Introduction

The production of physiologically active emanations by plant tissue in general, and especially by ripe fruit, has been demonstrated by epinastic responses of tomato and potato leaves, by inhibition of seed germination and potato sprouting, by the triple response of etiolated legume seedlings, and by effects on fruit respiration and softening. In all cases which subsequently have been investigated chemically, the active substance has been shown to be ethylene. This is now so well established and generally accepted that demonstration of the activity of an emanation by any of the above methods is a very strong indication that ethylene is among the gases evolved [(**18, 19, 20, 21**) and review by BIALE and SHEPHERD (**4**)].

A number of workers have found that the production of ethylene by various fruits is associated with the increase in rate of carbon dioxide output which is known as the climacteric rise in fruit respiration. HANSEN (**7, 8**) and HANSEN and HARTMAN (**9**) found that the peak of ethylene production coincided closely with the climacteric peak in the pear. KIDD and WEST (**11**) report that the development of the substance in apple vapor which inhibits germination of pea and mustard seeds coincides with the climacteric rise. NELSON (**14, 15**) found that several varieties of apples show a pattern of varying ethylene content throughout storage life analogous to the course of carbon dioxide evolution. Subsequently (**17**) he compared the evolution of carbon dioxide and ethylene by the McIntosh variety. In this study it appeared that the peak of ethylene production occurred somewhat later than the peak of carbon dioxide output. On the other hand, for the banana (**14, 15, 16**) he found that the highest rate of ethylene evolution comes before the peak of carbon dioxide production, with a secondary peak afterward; evidence was presented that ethylene is consumed during the peak of carbon dioxide output.

DENNY and MILLER (**6**) obtained a positive epinastic response with young potato plants subjected to the emanations of avocado fruit, and recent studies on the respiration of the avocado (**2, 3, 22**) have shown that this fruit has a well-marked climacteric rise. It therefore became of interest to study the relation between production of active emanations and the course of respiration.

Materials and methods

Etiolated legume seedlings are markedly affected by minute concentrations of ethylene gas, showing the "triple response" which is defined by

KNIGHT and CROCKER (12) as a "change of negative geotropism to diageotropism, increased growth in thickness, and reduced rate of growth in length." That the response of the pea epicotyl varies with the concentration of ethylene, giving well-defined qualitative and quantitative responses at different levels, was shown by NELJUBOW (13). ROHRBAUGH (18) has shown that the etiolated Alaska pea responds to as little as 0.05 parts per million of ethylene in air, and this has been confirmed by work in progress in this laboratory. Accordingly, young etiolated pea seedlings of this variety were chosen as test plants for the present study; uniform lots can be produced easily at any time, and their sensitivity is about equal to potato or tomato leaf epinasty. The maximum response is produced by five to ten parts per million of ethylene (fig. 1).

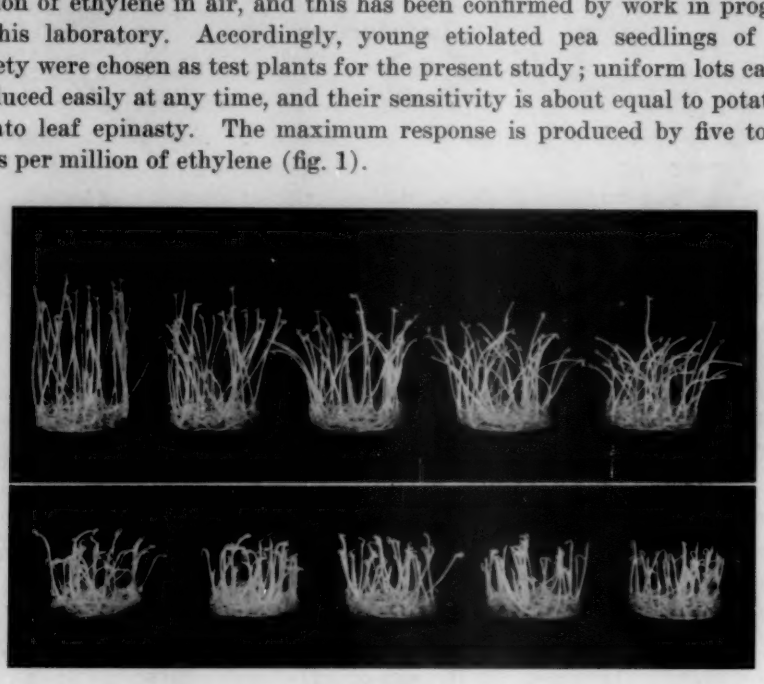

FIG. 1. The response of etiolated pea seedlings at 25° C. to known concentrations of ethylene gas in air. Top (from left): Control, 0.05, 0.10, 0.15, and 0.25 parts per million. Bottom: 0.50, 1.0, 2.5, 5.0, and 10.0 parts per million.

Test sets of peas were prepared by the following series of operations, all conducted in a room maintained at 25° C.: Lots of seed were soaked for seven hours in distilled water and then placed on wet filter paper in ten-centimeter Petri dishes, fifty seeds per dish. After two days, they were examined and reduced to the most vigorous thirty-five seedlings per dish. At this time the dish covers were removed, and the peas were watered with a saturated solution of calcium sulphate and placed in a humid atmosphere in the dark. On the sixth day, the shoots were about four centimeters high and the roots well developed. At this time they were ready for use, but if not used at once they could be kept in the dark at 5° C. until needed (as long as two weeks), resuming normal growth on return to 25°. Saturated calcium sulphate solution was used for watering the seedlings because it provided an easily prepared medium of constant composition which gave significantly better results than distilled water.

The test for active emanation was conducted by passing the air stream from a jar of fruit into the 25° room, through a water bubbler to equalize temperature and humidity, and through a large metal can containing two dishes of pea seedlings. The growth of the test plants relative to the controls was not affected by the original temperature of the air stream, by change in the rate of flow of air (200 or 350 ml. per minute), or by the reduced oxygen content of a modified atmosphere. The duration of exposure of each set of test plants was two days, after which the peas were photographed and a fresh set placed in series with the fruit, the photograph being marked with the date of removal of the set shown. The fruit was handled and respiration determined by methods previously described (**2**). Three storage temperatures were used—5°, 15°, and 25° C. The air stream was not connected to the pea containers during fruit respiration determinations (four hours per day).

Results

Production of active emanation in relation to carbon dioxide evolution

Results under normal atmosphere.—In a preliminary experiment it was found that Fuerte avocados at 15° gave off a gas which, during the period of respiratory rise, induced the triple response in etiolated pea seedlings. A more detailed study of this behavior was undertaken with fruit stored at different temperatures. Twenty freshly picked mature fruits (four to five kilos fresh weight) of the Fuerte variety were stored in each jar, with three jars for the first experiment and two for the second, one jar being placed at each temperature studied. The fruit in the 15° and 25° chambers was subjected to an air flow of 350 ml. per minute, while that in the 5° room was at 200 ml. per minute. The first carbon dioxide determination was made the day after picking. The results of these experiments are shown in figure 2.

It readily can be seen that the agreement in respiratory trend of the fruit at corresponding temperatures in the two experiments is remarkably close. In both cases the respiratory magnitudes were the same, and the time lapse between minimum and maximum respiratory activity was three days at 25° and five days at 15°, although the rise started one day sooner in the second experiment. The rates of carbon dioxide evolution, however, are markedly different at the two temperatures, and the slopes of the ascending curves are twice as great at 25° as at 15°. At the peak of the climacteric rise, the temperature coefficient (Q_{10}) for the range of 15° to 25° was 1.84 in the first experiment and 1.77 in the second.

In the first experiment, the fruit at 25° (jar 31) did not produce enough of the active emanations through the second day in storage (March 24) to cause any response in the first set of test plants. One day later, an initial response was shown by a definite decrease in length growth of the second set. On the fourth day (March 26) the concentration of emanation had increased

sufficiently to cause marked thickening and horizontal growth of the second set of peas. At that time the fruit at 15° and 5° still gave no response, as shown in figure 3. The first evidence of a response from fruit at 15° (jar 25A) was observed in the third set on the fifth day (March 27), coinciding with the first stage of the rise in carbon dioxide evolution. This date marked the carbon dioxide peak for the fruit at 25°, and its maximum pea response was shown by this third set. On the eighth day (March 30), the respiration rate at 15° was at its maximum, as was the pea response (fig. 4, the fourth test set). On the other hand, the fruit at 25° had already passed its climacteric, and the pea response was significantly diminished. In the second

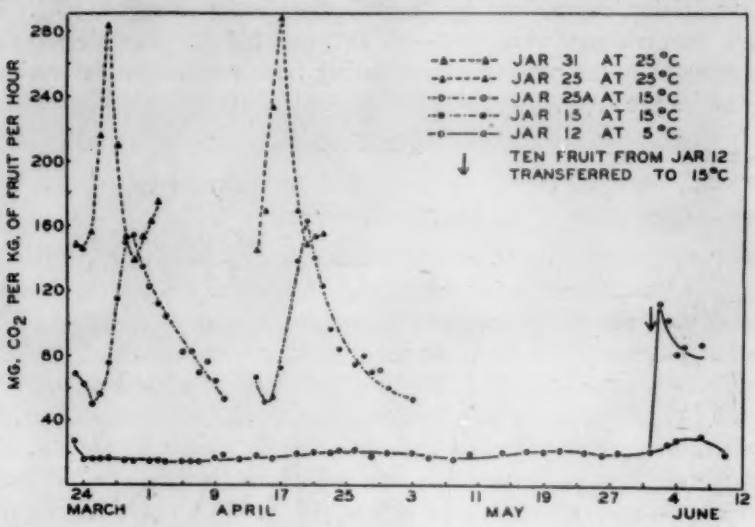

FIG. 2. Carbon dioxide evolution by Fuerte avocados stored in air at different temperatures.

experiment the same relationship between emanations and respiration was shown.

The analysis of fruit behavior at 5° presents some difficulties because of the very much reduced respiration rates. From a superficial examination of the curve, it would seem that the climacteric peak took place during the period of June 4 to June 7. This, however, is not in conformity with previously discovered facts and with new evidence to be introduced here. The climacteric rise must have taken place prior to June 1, because the fruit transferred to 15° on that day shows a descending curve of carbon dioxide evolution, with a maximum observed value much lower than that expected for this temperature. It was shown in a previous paper (3) that postclimacteric fruit will show this sharply descending curve when moved to a higher temperature, while preclimacteric fruit gives a typical increase to a maximum followed by a decrease in respiration. This contention will be supported further by an examination of the pea responses. Although at 5° (jar 12) the effect of avocado emanations on the growth of peas is less

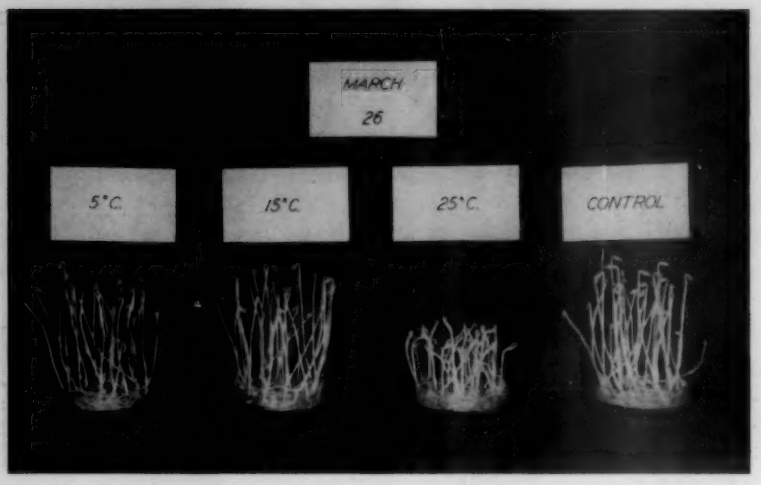

FIG. 3. The response of etiolated pea seedlings at 25° C. to emanations from Fuerte avocados stored in air at the temperatures indicated.

sharply defined, as early as April 9 the peas appeared slightly shorter than the controls. It was not until almost mid-May that marked horizontal growth was first noted. A maximum pea response was observed for the period from May 18 through May 22 (fig. 5), after which the response decreased gradually. A definite, though weaker, response persisted to the end of the experiment. The behavior of the fruit at 5° suggests that, under these conditions, the production of an active emanation may be a better index for the physiological changes characteristic of the climacteric than the rate of carbon dioxide evolution.

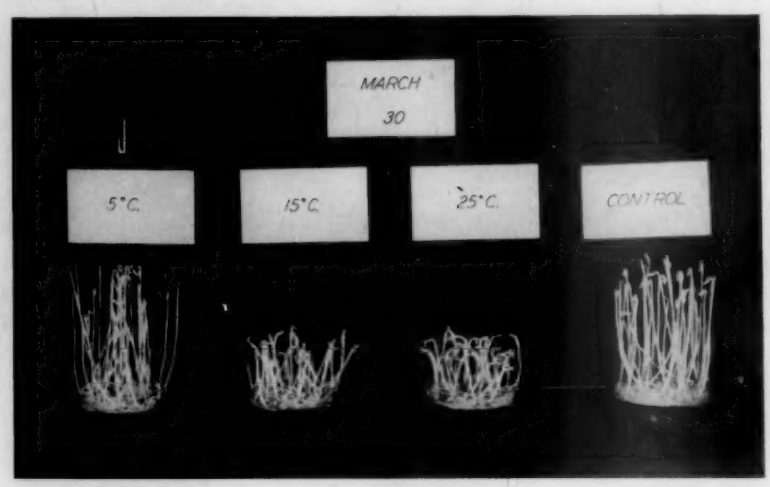

FIG. 4. The response of etiolated pea seedlings at 25° C. to emanations from Fuerte avocados stored in air at the temperatures indicated.

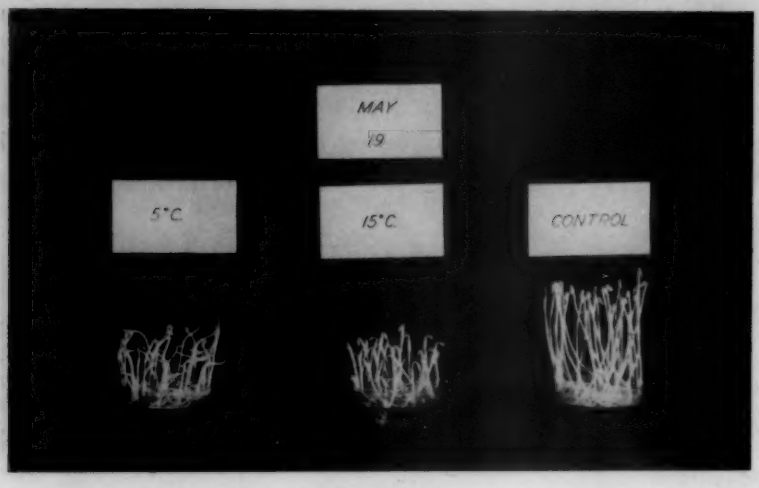

FIG. 5. The response of etiolated pea seedlings at 25° C. to emanations from Fuerte avocados stored at 5° C. in air and at 15° C. in a modified atmosphere (2.5 per cent. oxygen in nitrogen).

The activity of avocado fruit emanation was further verified by tomato leaf epinasty in one instance. On April 7, one avocado from jar 25A was transferred to 25° and placed in a jar with a tomato plant having four well-developed leaves. The carbon dioxide given off was absorbed in potassium hydroxide solution, and the oxygen was replenished from a bottle under the pressure of a constant water level siphon. The strong response produced in 24 hours is shown in figure 6.

An experiment similar to those described for the Fuerte avocado was performed with Nabal, one of the leading summer varieties in California.

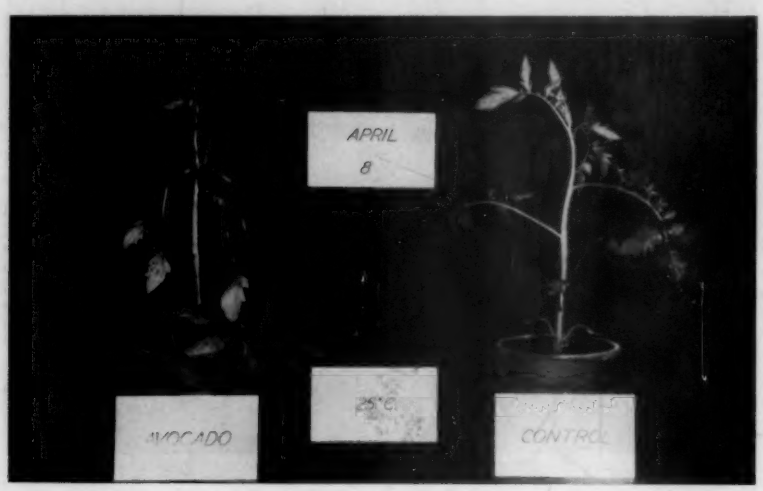

FIG. 6. Tomato leaf epinasty induced by emanation from a Fuerte avocado fruit.

Nine freshly picked mature fruits (four to five kilos fresh weight) were used for each container. In this case the fruit was placed at 15° and 25° only. The trend of respiration was very similar to that of the Fuerte, although the respiratory activity was somewhat lower, particularly before the climacteric rise. At 15° the minimum carbon dioxide values were about fifty per cent. less than in the case of Fuerte, and it took twice as long for the maximum to be attained. The reaction of the peas showed, however, that the trend of fruit emanation was essentially the same for the Nabal avocados as for the winter variety, with the maximum response coinciding with the highest rate of carbon dioxide production.

EFFECTS OF LOW ATMOSPHERIC OXYGEN.—In experiments with different oxygen tensions in relation to respiration and storage (3), it was found that

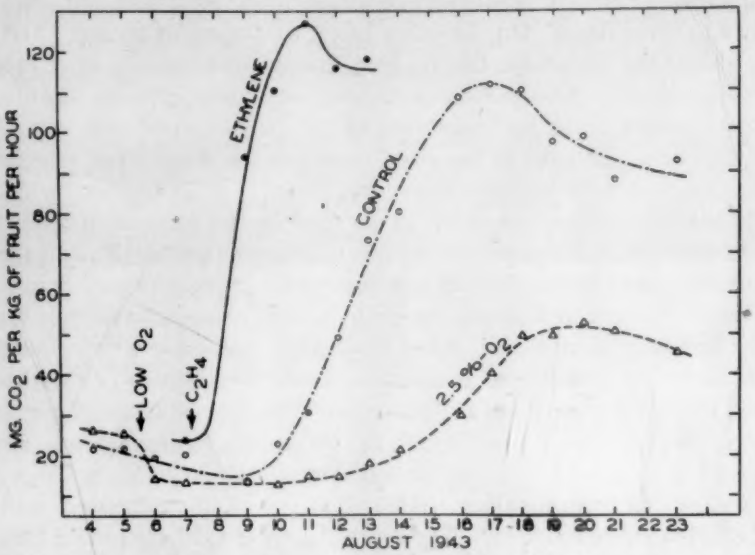

FIG. 7. Carbon dioxide evolution by Nabal avocados at 15° C. under treatment with ethylene gas (200 p.p.m.), with a modified atmosphere (2.5 per cent. oxygen in nitrogen), and in air (control). Treatments started at the times indicated by arrows.

the climacteric rise can be delayed and its magnitude reduced by subjecting the fruit to a reduced oxygen content of the atmosphere. The question arose, therefore, as to what effect this might have on the production of active emanations. It can be seen from figure 7 that the peak in respiration of Nabal fruit at 15° under 2.5 per cent. oxygen (in nitrogen) was reached four days later than in the control, and its value was decreased by more than 50 per cent. On August 13 the peas subjected to the air stream from the control fruit showed horizontal growth, while those connected to the fruit under low oxygen were only slightly reduced in length. Maximum pea response was reached by August 16 for the control and by August 20 to 23 for the modified atmosphere set. These dates correspond with maximum respiratory activity. Results obtained earlier with Fuerte under the

same conditions were similar, the reduction in carbon dioxide output being even more striking. Although respiration is markedly reduced, sufficient active emanation is produced under low oxygen to give the maximum pea response (fig. 5, 15°).

Treatment of avocados with ethylene gas

Many plant materials are known to show a physiological response to applied atmospheric ethylene. This particularly applies to mature fruit in its pre-climacteric state, ethylene causing more rapid onset of the climacteric rise and the related ripening processes (9). The treatment of avocados has been considered by other workers; Harvey (10) and Adriano *et al.* (1) treated fruits of the Baldwin, Cardinal, Lyon, and Pollock varieties with 1000 parts per million of ethylene in air, securing softening and good flavor in two to three days. On the other hand, Chace and Church (5) treated avocados of the Blakeman, Challenge, Colorado, and Solano varieties at high temperature with 2000 parts per million with unsatisfactory results. The latter, however, must be discounted as the fruit studied was not commercially mature. In none of the cited cases was the respiration rate followed in conjunction with ethylene treatment.

Nabal fruits from the same lot as used in the emanation study above were treated with 200 parts per million of ethylene gas at 15°, and the effect of the treatment on respiration was observed. During twenty hours a day, the fruit was kept in a closed system in which carbon dioxide was absorbed by an excess of potassium hydroxide solution, and oxygen was added from a bottle under pressure of a constant water level siphon. Ethylene was added to the fruit jar from a gas burette (2 ml. per 10 liters of free space). During the remaining four hours, the potassium hydroxide was removed, and carbon dioxide-free air was passed through the jar at 350 ml. per minute for respiration determination. After this period, the potassium hydroxide and ethylene were renewed. The control jar was subjected to an air stream throughout the experiment. In a subsequent experiment two controls were used, one in a closed system as described above and the other subjected to an air stream. No significant difference due to confinement in the closed system was observed.

The result of ethylene treatment is seen in figure 7. The climacteric rise was initiated immediately after ethylene was applied, and the respiration rose rapidly, reaching its peak in four days. The fruit was found to be fully softened on the following day. On the other hand, the control fruit started its rise four days later, required five to six days to reach the peak, and softening was correspondingly delayed. Furthermore, the control lot was variable in softening time, while the ethylene-treated fruit softened uniformly and was of somewhat better quality.

Summary

The carbon dioxide evolution of Fuerte and Nabal avocados was studied at three different temperatures. At 15° and 25° C. a very pronounced climacteric rise was observed, while at 5° C. there was no well-defined increase.

Associated with the climacteric rise was the production of an active emanation, presumably ethylene, as shown by the triple response of etiolated pea seedlings. Although there was no obvious climacteric rise at 5°, a cycle of emanation production was observed, after which the fruit showed a post-climacteric carbon dioxide response when transferred to a higher temperature.

Treatment of pre-climacteric fruit with ethylene caused an earlier onset of the climacteric rise and more uniform softening.

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PHYSICAL CHARACTERS OF THE SKIN IN RELATION TO APPLE FRUIT TRANSPIRATION[1]

S. A. Pieniazek

(WITH ONE FIGURE)

Wilting of the skin is one of the main storage problems with some varieties of apples. It occurs when the fruit loses between 7 and 10 per cent. of its original weight (**2**). The loss in weight is due principally to the loss of water in transpiration and to a lesser extent to the loss of carbon in the respiration process. A thorough understanding of the factors influencing transpiration is therefore of great value from practical as well as theoretical points of view.

The rate of transpiration of fruits is influenced by external and internal factors. While considerable work has been done on external factors (**9**) such as temperature, humidity and movement of the air, not much is known about the internal factors or those contributed by the fruit itself, influencing the rapidity of water loss. Magness (**6**), Hinton (**4**), and Pieniazek (**10**) investigated the effect of maturity on the rate of transpiration of apples. More attention, however, was given to the physical characters of the skin.

W. H. Smith (**13**) found that the thickness of cuticle is not necessarily positively correlated with the rate of transpiration of Cox Orange Pippin apples. Bell (**1**) made a detailed study of the protective layers of Red McIntosh apples, while Markley (**7**) and Hulme (**5**) devoted their attention to the layer of waxy coating on the surface of the cuticle. This layer is very thin in comparison with the thickness of the cuticle and it is difficult to measure even under high magnification. It differs from cuticle in its chemical composition and its physical properties (**7**). E. R. Smith (**12**), Robinson (**11**) and Marshall *et al.* (**8**) on the Pacific Coast demonstrated that severe washing treatments greatly increased the rate of water loss from apples, by dissolving the layer of surface wax. Clements (**3**) found the apple lenticels to be open while on the tree, and gradually closed in storage. He made no attempts to measure and compare the rates of lenticular and cuticular transpiration.

Material and methods

The fruits used for the 1941–1942 season experiments were grown in the experimental orchards of Cornell University, Ithaca, New York, and for the 1942–1943 season in the R. I. State College orchards at Kingston, R. I. The rate of transpiration was measured by weighing fruits on a sensitive torsion balance and the loss in weight was assumed to be due to loss of water. No correction was made for the loss of carbon in the respiration process.

Free hand sections of the skin perpendicular to the surface were prepared and stained by an alcoholic solution of Sudan III. The thickness of

[1] Contribution no. 631 of the Rhode Island Agricultural Experiment Station, Kingston, Rhode Island.

cuticle was measured under a microscope with the aid of a sliding ocular micrometer.

The following method was employed for the approximate determination of lenticular transpiration. A number of apples were weighed individually for a few days in order to determine the rate of transpiration for each fruit. They were divided then into three lots in such a way that the rate of transpiration of each lot was the same.

The differential treatment was now given. The fruits of lot 1 were left as check. The fruits of lot 2 had their lenticels blocked with melted paraffin,

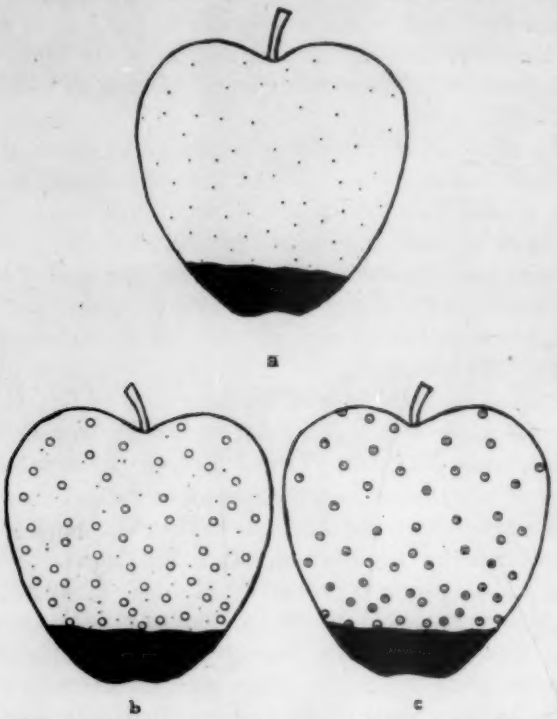

FIG. 1. Blackened areas and circles—covered with paraffin. Points—lenticels. Symbols: a = check; b = paraffin between lenticels; c = lenticels covered.

which was deposited in small drops by means of a thin camel hair brush. Since paraffin covered also some surface around the lenticels, the fruits of lot 3 were covered with about the same number of paraffin drops deposited on the cuticle between lenticels. Thus the difference in water loss between lot 3 and lot 2 was assumed to be due to the lenticular transpiration. When divided by the transpiration rate of the check fruits it gave the value of lenticular transpiration in percentage of total transpiration.

In the course of the work it appeared impossible to cover every lenticel separately toward the calyx end of the fruit, where they are numerous. The method was slightly modified. All apples at the start had the calyx end covered with paraffin, and their transpiration rate measured, after which

they were divided into three lots and given differential treatment. Figure 1 illustrates the final method.

The influence of waxy coating of apples on the transpiration rate was studied by comparing water loss of normal fruits with those from which the wax was removed. Two lots of fruits were usually selected on the basis of individual weighings over a few days' period. This was done to have the lots losing water at the same rate. The wax was taken off from one lot and increase in transpiration rate was measured by comparison with the check lot.

There is no method by which all waxy coating can be taken off with negligible injury to the fruit. In this study the fruits were simply wiped off with clean cheesecloth. No effort was made to show what portion of the waxy layer was removed in this way.

Results and discussion

Thickness of cuticle

Four varieties widely differing in their transpiration rates were selected for this study. Ten fruits of each variety were taken out of storage on February 21, 1941, kept for five days in a constant temperature room at

TABLE I

Thickness of cuticle in relation to transpiration rate of apples

Variety	No. of fruits	Initial weight	Loss of weight during 5 days	Cuticle thickness
		gm.	*gm.*	*microns*
McIntosh	10	1625.42	32.05	37.2 ± 2.20
Baldwin	10	1620.18	39.10	39.4 ± 2.00
Golden Del.	10	1619.20	45.32	30.3 ± 1.91
Rhode Island Greening	10	1623.41	30.80	39.5 ± 1.97

25° C. and then the sections were prepared. Four sections were taken from each fruit, and four measurements from each section, in order to find the average cuticular thickness. This means that 160 measurements were taken for each variety. The mean values were then calculated and they are given in table I together with their standard errors.

The data do not show any definite relationship between the transpiration rate of four varieties and the thickness of their cuticles. Golden Delicious is characterized by a relatively thin cuticle and a high transpiration rate, but Rhode Island Greening and Baldwin have cuticles of approximately the same thickness and vary widely in their transpiration rates.

In the same season two lots of McIntosh apples were selected. Fruits of one lot were found to transpire at a rate 30 per cent. higher than the fruits of the other lot. Average cuticular thickness of the two lots differed but little, measuring 36.7 and 37.3 microns respectively. No relationship between the thickness of cuticle and the rate of transpiration could be thus established between individual fruits of the McIntosh variety.

LENTICELS

The data obtained in November, 1941, are given in table II. They show considerable differences between varieties as to the intensity of lenticular transpiration. In the variety Turley, which has very few lenticels, they account only for about 8 per cent. of the moisture lost in transpiration. In Golden Delicious, lenticels are large and numerous and they account for over 20 per cent. of the total transpiration.

TABLE II

RÔLE OF LENTICELS IN THE TRANSPIRATION OF APPLE FRUITS

LOT	INITIAL WEIGHT	TREATMENT*	TRANSPIRATION DURING 24 HOURS		LENTICULAR TRANSPIRATION LOT 2—LOT 1	LENTICULAR TRANSPIRATION IN PERCENTAGE OF TOTAL TRANSPIRATION
			BEFORE TREATMENT	AFTER TREATMENT		
	gm.		*gm.*	*gm.*	*gm.*	%
			Golden Delicious			
1	1028.18	A	5.80	3.33		
2	1104.94	B	5.80	4.40	1.07	21.4
3	1115.93	Check	5.81	5.00		
			Baldwin			
1	1052.55	A	3.41	1.96		
2	1086.08	B	3.41	2.61	0.65	20.9
3	1052.38	Check	3.41	3.10		
			Turley			
1	1055.94	A	3.74	2.49		
2	1064.19	B	3.74	2.74	0.25	7.8
3	1003.16	Check	3.74	3.22		
			Red Canada			
1	980.23	A	3.52	2.22		
2	920.61	B	3.53	2.81	0.59	18.9
3	951.65	Check	3.52	3.12		

* Treatment A: Lenticels covered with paraffin drops. Treatment B: paraffin drops deposited on the skin between lenticels.

The number of lenticels in the skin of the apples is very small as compared with the number of stomata per unit area of leaves and yet quite a considerable amount of water is lost through them. It is obvious that these structures are very effective in the transpiration process. Since, however, the number of lenticels is small, they are not decisive factors in the transpiration of a fruit as a whole. About five to ten times more water is lost directly through the cuticle than through lenticels.

It should be emphasized that the number and appearance of lenticels alone give no indication as to the intensity of the potential rate of fruit

transpiration. A comparison of two varieties, Turley and Canada Red, made in November, 1941, serves as a good example. The former has less than 500 small, inconspicuous lenticels per fruit, the latter is characterized by numerous—over 1500 per fruit—and large lenticels but (in spite of that) the transpiration rates of these two varieties do not show any great difference.

Anatomical studies and some physiological experiments (3) indicate that lenticels are rather widely open early in the fall but they close gradually at harvest time and later in storage. No actual transpiration measurements were, however, reported.

In order to test the hypothesis, Delicious, Golden Delicious, and Baldwin apples were harvested on October 10, 1942, and their lenticular transpiration measured. The fruits were kept in the laboratory in a dry and warm atmosphere for a month and weighed every week. During this time considerable desiccation took place and gradual decrease of lenticular transpiration could be expected if the lenticels really close with time. The results obtained are given in table III. They seem to show a slight decrease in lenticular transpiration when expressed as a percentage of total transpiration. The change is rather small and does not suggest any appreciable change in the degree of lenticular opening.

TABLE III

INFLUENCE OF TIME FACTORS ON THE LENTICULAR TRANSPIRATION OF APPLE FRUITS

VARIETY	NO. OF FRUITS USED	LENTICULAR TRANSPIRATION IN PERCENTAGE OF TOTAL TRANSPIRATION			
		1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK
		%	%	%	%
Baldwin	30	17.2	15.3	16.2	13.0
Delicious	30	25.2	25.0	24.3	20.6
Golden Delicious	30	18.3	15.2	17.0	16.6

Some fruits of the same varieties harvested on October 10, 1942, were stored in the cold room at approximately 40° F. until January 10, 1943, when their lenticular transpiration was measured. The data are similar to those given in table III. During the 3 months of storage the relative lenticular transpiration did not change appreciably in comparison with the data obtained directly after harvest. These two experiments indicate that there is little change in relative lenticular transpiration rate after the apples are harvested in the fall.

RUSSETING

Surface russeting, as caused by a combination of climatic factors and spray materials, is responsible for increasing greatly the rate of transpiration of affected fruits. Such apples invariably shrivel first on the russeted side. In October, 1942, the rates of transpiration of russeted Delicious, Golden Delicious, and Baldwin apples were compared with those of normal fruits at room temperature. The results are given in table IV.

TABLE IV

INFLUENCE OF RUSSETING ON THE RATE OF TRANSPIRATION OF APPLE FRUITS

VARIETY	NO. OF FRUITS USED	EXTENT OF RUSSETING*	WEIGHT OF FRUITS	TRANSPIRATION RATE	RELATIVE TRANSPIRATION RATE
			gm.	*mg./kg./hr.*	
Baldwin	20	20%	3751.0	246	148
"	20	None	3763.5	166	100
Delicious	20	25%	3851.3	220	166
"	20	None	3865.2	132	100
Golden Del.	20	15%	3002.1	285	108
"	20	None	3015.7	264	100

* Estimated.

It was shown that russeting greatly increased the rate of transpiration of Baldwin and Delicious apples, but only slightly that of Golden Delicious fruits. It is to be added, however, that there were two different types of russeting. The former varieties showed more abundant and deeper cork formation with numerous cracks present. The latter were covered with superficial cork and were characterized by lack of deeper cracks.

WAXY COATING

Some preliminary experiments on the influence of waxy coating of apple skin on the rate of transpiration were made in the season of 1941–1942 and extensively studied in the fall and winter of 1942–1943. Baldwin, Golden Delicious, McIntosh, Rhode Island Greening, and Tompkins County King were selected for the study, 10 to 20 pairs of fruits being used for each experiment. In all, more than 1500 fruits were weighed individually. They were harvested early and late in the harvest season, the wax layer studied at picking time, and later in storage.

It was found that the varieties studied here can be arranged in the following order: Golden Delicious, McIntosh, Baldwin, Tompkins County King and Rhode Island Greening, the first having the least and the last the most effective wax layer in checking transpirational losses. Approximate average increases in transpiration resulting from removing the wax layer directly after harvest were 8, 13, 18, 20, and 25 per cent., respectively. The wax

TABLE V

PERCENTAGE INCREASE IN TRANSPIRATION RATE OF APPLES AT ROOM TEMPERATURE AS CAUSED BY REMOVAL OF NATURAL WAX

PICKING DATE	SEPT. 11	SEPT. 25	OCT. 3	OCT. 17	OCT. 28
Variety	%	%	%	%	%
Baldwin	9	11	15	20	19
Golden Del.	5	6	6	7	9
McIntosh	10	13	14	16	13
Rhode Island Greening	15	28	35	38	42
Tompkins Co. King	13	15	17	28	34

layer on the surface of the fruit increased as maturity approached and later when this stage was passed. This is shown by the fact that the removal of the wax layer increased the transpiration rate to a higher degree in late than in early picked fruits. Table V gives the approximate increase in water loss as caused by removal of surface wax from the fruits picked at certain time intervals.

The data given in table VI show that the wax layer increases in storage after harvest.

An apple that has been wiped off looks bright and shiny as contrasted with the rather dull untreated one. The difference disappears in a few days probably because a new layer of natural wax forms on the treated fruit. The new layer of wax is, however, not so effective in diminishing water loss and the difference in transpiration rate that results after wiping remains almost constant for a long time.

TABLE VI

PERCENTAGE INCREASE IN TRANSPIRATION RATE AS CAUSED BY REMOVAL OF WAX FROM FRUITS STORED AT 40° F.

VARIETY	PICKING DATE	EXAMINATION DATE			
		OCT. 25	DEC. 23	FEB. 22	MAR. 19
		%	%	%	%
Baldwin	Oct. 17	18	16	19	22
Golden Del.	Oct. 18	7	8	8	10
McIntosh	Sept. 15	13	13	15	14
Rhode Island Greening	Sept. 23	43	58	70	88

The mechanical wiper as it is used for removal of spray residues in Rhode Island and some other states is almost as efficient in removing the wax layer from the surface of the apple as the hand wiping. Transpiration rate of Baldwin apples was increased by 17 per cent., and that of Rhode Island Greening apples by more than 30 per cent., as a result of running them through a wiping machine.

Conclusions

1. No correlation was found between the thickness of the cuticle and the rate of transpiration of apple fruits either between individual fruits within the McIntosh variety or between the four varieties studied.

2. It was found that lenticular transpiration accounts for from 8 to over 25 per cent. of total transpiration of apple fruits of the varieties studied. Lenticels appear to be more effective in transpiration directly after harvest than later in storage, but the change was not found to be great. The number and size of lenticels is in no way correlated with the rate of total transpiration of apple fruits.

3. Surface russeting, characterized by abundant cork formation and deep cracks was found to increase water loss greatly.

4. The layer of waxy coating on the surface of the skin was found to be of great importance in diminishing water losses from apple fruits. Apple

varieties differ in the amount of wax deposited on the surface of the cuticle. Varieties with large deposits of wax, as Rhode Island Greening and Tompkins County King, lose water slowly. Golden Delicious is characterized by a very insignificant layer of wax and by a very high transpiration rate. A considerable amount of the surface wax can be taken off by wiping the fruit with a rag or by running it through a mechanical wiper. These operations result in an increase in the rate of transpiration that will not be suppressed by the new layer of wax forming on the surface of the skin.

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LIGHT AND THE ELONGATION OF THE MESOCOTYL IN CORN

LEWIS H. FLINT

In reporting the results obtained in a study of the mesocotyl of *Avena sativa,* WEINTRAUB and MCALISTER (**12**) directed further attention to this interesting plant structure. The early experiments of COLLINS (**2**) had indicated that in maize the potential extensibility of the mesocotyl in darkness was a varietal characteristic evidencing suitability for agricultural use in different habitats. The Chinese type, grown on shallow soils, possessed mesocotyls with a minimum degree of extensibility; the central and eastern United States type, grown on deeper soils, had mesocotyls with a medium degree of extensibility; while the Hopi Indian type, planted deep in drier soils, had mesocotyls with the maximum degree of extensibility. A further differentiation within these three types concerned the physiology of the organ. While in general the mesocotyl appeared to function primarily to insure the establishment of the root system at a suitable distance below the soil surface, thus appreciably discounting the significance of experiments on depth of planting, the mesocotyl of the Hopi Indian type of maize persisted and functioned somewhat as a tap root, thereby contributing to the possible culture of the plant under semi-desert conditions. KEMPTON (**10**) studied the inheritance of mesocotyl length in this latter type.

While such a variability within a species makes analogy precarious, it perhaps may be assumed tentatively that the general function of the mesocotyl is related to the disposition of the root-system below the surface; that is, in darkness. The potential extensibility of the mesocotyl in our common grass and grain plants is in appreciable excess of its normal extension under field conditions and the inhibition of the mesocotyl therefore may be assumed to be effected by external factors. The present day vogue and viewpoint would suggest the inhibitory action as associated with the downward movement of substances from the adjoining apical structures. Of particular interest in conjunction with such a postulated movement is the action of light upon these adjoined structures.

In so far as quality of light is concerned, the phototropic response of plants appears to be fairly well restricted to the shorter wave-length region —4000 A to 5400 A. While all of the protoplasmic pigments absorb in this region, the existence of phototropism in chlorophyll-free carotin-containing fungi, *e.g.,* Pilobolus (**3**), appears to indicate an association of carotenoids with phototropism. Explanations of phototropism in terms of the action of light on "growth substances" have involved changes in distribution, direct inactivation, and the production of inhibitors. In some measure these explanations have been carried over into the interpretation of data indicating the effectiveness of the same short wave-length radiations in bringing about stunting or the general inhibition of elongation. This step has been a natural outgrowth of considerations proceeding from a horizontal to a

vertical incidence of the short wave-length radiation. Yet, however interpreted, there has been considerable evidence that radiation of the shorter wave-length range, 4000 A to 5400 A, exerted an inhibitory action upon elongation processes in comparison with an implied, assumed, or demonstrated inactivtiy of the longer wave-length radiation. BURKHOLDER, for example (**1**), has brought together such evidence. In the marked stunting of plants at high altitudes the situation has appeared complicated by low temperatures, but both the stunting and the abundance of anthocyanin pigments have been attributed to shorter wave-length radiation. INGE and LOOMIS (**8**) reported that the elongation of the mesocotyl in corn seedlings was stopped by exposing coleoptile tips to light, particularly to ultra-violet light. In studies of quality of light in relation to the germination of light-sensitive lettuce seeds it was found that low intensity shorter wave-length radiation had an "inhibiting effect" as contrasted with the "promoting effect" of low intensity longer wave-length radiation (**4, 5, 6**). While these results had no obvious relation to elongation processes, they indicated opposite effects attributable to "blue" and "red" radiation and associated the shorter wave-length radiation with an inhibitory effect. In studies of the responses of the simple leaves of young bean plants to "green" and "red" light it was found that following decapitation there was a renewal of development under red light—including an elongation of the petioles—in contrast to the absence of any such development under green light (**7**).

On the other hand, there has been evidence to support the conclusion that the longer wave-length visible radiation was effective in the inhibition of elongation and growth. Studies by WENT (**13**) of the responses of pea plants in relation to quality of light gave results interpreted as such evidence. Experiments on quality of light in relation to the growth of beans led ROHRBAUGH to conclude that the longer wave-length radiation was the more effective in reducing elongation (**11**). The results of studies by JOHNSTON (**9**) and by WEINTRAUB and MCALISTER (**12**) on the responses of Avena seedlings to low intensity radiations were interpreted as evidence of an inhibitory effect of red light on mesocotyl elongation. The citation of results favoring each of the foregoing conclusions might be continued at some length.

The experiments here described gave results which appear to lead to interpretations directly the opposite of those derived by WEINTRAUB and MCALISTER. There is, of course, no particular reason why one need insist that all plants or all mesocotyls behave alike with respect to red light and blue light. Some samples of lettuce seed, for example, are sensitive to light while others are not, and it is possible that such seed differences may be correlated with processes other than or continuing beyond germination. In any event one may be certain and appreciative that plants themselves continue to behave in an intriguing manner, yet unmotivated by any consciousness of controversial issues.

Experimentation

To obtain some sort of background for the evaluation of possible temperature effects associated with exposures to light, a series of tests was carried out in which grains of corn were germinated on saturated absorbent paper towelling in closed tin cans placed at different temperatures. The grains were selected for uniformity of size and were of the variety Funk's Disease Free—procedure followed in all tests with corn. The results obtained have been brought together in table I.

The results given in table I were interpreted as indicating that small differences in temperature might have an appreciable effect upon mesocotyl elongation. For example, in comparing the mesocotyl lengths at 25° and 30° C., at the end of four days an increase in temperature from 25° to 26° might be presumed to have caused a pronounced increase in elongation, while

TABLE I

RESULTS OBTAINED IN A STUDY OF THE ELONGATION OF THE MESOCOTYL OF CORN IN DARKNESS AT DIFFERENT TEMPERATURES. HUMIDITY, 100 PER CENT. VALUES IN MILLIMETERS TO THE NEAREST WHOLE NUMBER

TEMPERATURE	AVERAGE LENGTH OF MESOCOTYL (12 IN EACH SET)			
	AFTER 4 DAYS	AFTER 8 DAYS	AFTER 12 DAYS	AFTER 16 DAYS
°C.	mm.	mm.	mm.	mm.
45	0	0	0	0
35	14	67*	70	70
30	34	79	95*	95
25	6	85	103	122*
20	0	38	.....	80
15	0	0	6	29

* Leaves mostly emergent from the coleoptiles.

at the end of sixteen days a similar increase in temperature might be presumed to have caused an apparent decrease in elongation. Yet the results also indicated that the higher the temperature of germination the more quickly was there an emergence of the leaves from the coleoptiles, accompanied by a cessation of the elongation of the mesocotyl. For example, a difference of five degrees (as 25° to 30°, or 30° to 35°) appeared to hasten by about four days the time of emergence of the leaves and the cessation of mesocotyl elongation.

While the temperature experiments were in progress a reconnaissance series of tests was carried out to study light intensity and quality as factors inhibiting mesocotyl elongation. In these tests selected grains were placed on moist blotting paper in moist chambers placed at different distances from the light sources. Opaque chambers were used as controls. The results obtained have been brought together in table II.

The results given in table II were interpreted as indicating that a very low light intensity, as compared with sunlight, was sufficient to effect a

TABLE II

RESULTS OBTAINED IN A STUDY OF INTENSITY AND QUALITY OF LIGHT AS FACTORS INHIBITING THE ELONGATION OF THE MESOCOTYL OF CORN. VALUES ARE RELATIVE MESOCOTYL LENGTHS, AVERAGES FOR TWELVE PLANTS

ILLUMINATION IN FOOT-CANDLES (WESTON PHOTRONIC CELL)	SOURCES OF LIGHT		
	NEON TUBE (RED LIGHT)	DAYLIGHT MAZDA (WHITE LIGHT)	MERCURY TUBE (BLUE LIGHT)
fc			
0	100.0	100.0	100.0
5	24.0	25.4	17.2
10	20.0	14.4	10.8
25	20.0	8.4	.....
50	11.2	5.2	.....
100	6.8	2.4	.....

marked slowing down of the elongation of the mesocotyl in corn. While the shorter wave-length radiation appeared to be the more effective, it was recognized that wide wave-length ranges were involved and that temperature differences were not controlled.

A further series of tests was then carried out to study more carefully the relative elongation of the mesocotyls in blue light as compared with red light. Mazda lamps were used in conjunction with red and blue cellophane filters. Moist chambers with grains on moist blotting papers were placed inside a triple-glass-walled refrigeration unit, the lamps and filters being placed outside. Light intensities were established with a photronic cell and the temperature was constant at 18° C. The results have been brought together in table III.

While it was recognized that the photronic cell was not equally sensitive to red and blue light, it was considered that, using the same instrument, the results should have run parallel except as modified by quality of light. The results obtained were interpreted to signify that blue light was more effective than red light in slowing down the elongation of the mesocotyls. Yet in centering attention upon quality of light the effectiveness of red light as compared with darkness had been neglected. It became of interest, therefore, to carry out a series of tests designed to correlate the results given in

TABLE III

RESULTS OBTAINED IN A STUDY OF THE RELATIVE ELONGATION OF MESOCOTYLS OF CORN IN BLUE LIGHT, AS COMPARED WITH RED LIGHT, AT DIFFERENT INTENSITIES. TEMPERATURE, 18° C. VALUES BASED ON AVERAGES FOR 12 PLANTS

ILLUMINATION	RELATIVE ELONGATION OF MESOCOTYLS OF CORN IN BLUE LIGHT AS COMPARED WITH RED LIGHT AT THE SAME PHOTRONIC CELL VALUE, TAKEN AS 100
fc	
3	85.3
5	74.8
10	61.2
30	55.8

TABLE IV

RESULTS OBTAINED IN A STUDY OF THE ELONGATION OF THE MESOCOTYLS OF CORN UNDER VARIOUS CONDITIONS OF ILLUMINATION AND AT VARIOUS STAGES OF DEVELOPMENT. VALUES REPRESENT LENGTHS IN MILLIMETERS, AVERAGES OF 12 PLANTS. TEMPERATURE, 18° C. HUMIDITY 100 PER CENT.

TIME OF MEASUREMENT	ILLUMINATION			
	RED LIGHT AT 3 FC	BLUE LIGHT AT 3 FC	RED LIGHT AT 3 FC PLUS BLUE LIGHT AT 3 FC	DARKNESS
	mm.	*mm.*	*mm.*	*mm.*
After 12 days	13.54	5.61	9.625	14.22
After 22 days	16.45	9.38	14.83	39.21
After 36 days	.....	.....	.....	59.20

the foregoing tables. The methods used were similar to those indicated for the tests just described. The results obtained have been brought together in table IV.

The results given in table IV were considered as particularly suggestive in two respects. In the first place, at the time of the first set of measurements—after 12 days—the coleoptiles were practically all intact, whereas at the time of the second set of measurements the leaves were emergent from the coleoptiles in the plants under red light and blue light but not in the plants in darkness. The suggestion here was that red light and darkness were equally ineffective in inhibiting the elongation of the mesocotyls up to the time of emergence of the leaves. Yet, in the second place, the measurements with red light superimposed upon blue light suggested that red light was effective in offsetting or obscuring the inhibiting action of blue light. The methods and materials seemed well adapted to a special test of the first suggestion. Provision was made for the inclusion of a larger number of seeds and seedlings in order to facilitate statistical analysis of the data. The results obtained are presented in table V.

The results given in table V were interpreted as evidence that red light alone had no effect upon the elongation of the mesocotyl in corn up to the date of the emergence of the leaves from the coleoptiles while blue light alone had an inhibiting effect during this period.

The second suggestion arising from considerations of the values given

TABLE V

RESULTS OBTAINED IN A STUDY OF THE EFFECTS OF RED LIGHT, BLUE LIGHT, AND DARKNESS UPON THE ELONGATION OF THE MESOCOTYLS OF CORN. TEMPERATURE, 18° C. HUMIDITY, 100 PER CENT. MEASUREMENTS IN MILLIMETERS, AVERAGES OF 20 PLANTS, WITH PROBABLE ERRORS

TIME OF MEASUREMENTS	ILLUMINATION		
	RED LIGHT AT 30 FC	BLUE LIGHT AT 30 FC	DARKNESS
Emergence of leaves from coleoptiles in red light	11.57 ± 1.14	6.53 ± 0.69	11.7 ± 1.43

in table IV, that of red light counteracting the effect of blue light, seemed to accord a positive effect to red light. Yet the results given in table V discounted such an activity. One might guess that reflection or interference phenomena might be involved, but interpretation seemed complicated and precarious and no further tests were carried out.

Discussion

The experiments in darkness appeared to indicate that in corn the rate of elongation of the mesocotyl up to the time of the emergence of the leaf blade from the coleoptile was fundamentally associated with temperature and that no mesocotyl elongation took place following such emergence. The rate of elongation of the leaf within the coleoptile was evidenced as even more sensitive to temperature changes, and the higher the temperature the shorter was the period from seed germination to the time of leaf blade emergence from the coleoptile, and the attendant cessation of mesocotyl elongation. Depending upon the temperature, therefore, was the important consideration of the time during which other factors, as light, might be impressed and tested. Depending upon the relation of the growth temperature to the optimal growth temperature was the nature of any internal temperature effect associated with exposure to light.

The experiments in light appeared to indicate a high degree of sensitivity to blue light on the part of the coleoptile while this organ remained practically insensitive to red light. The blue light appeared to have a slowing-down action on the rate of elongation of the coleoptile without exerting a corresponding effect upon the leaf blade which it enclosed, and in consequence blue light shortened the period to leaf blade emergence, and caused the cessation of mesocotyl elongation. In addition, the coleoptile in blue light appeared to supply to the subtending mesocotyl a substance which slowed down its rate of elongation. This inference was based upon (1) the evidence of a slowing-down factor in the coleoptile exposed to blue light; (2) the obvious presence of pigments absorbing blue light in the coleoptile; and (3) the apparent absence of such pigments in the mesocotyl.

The foregoing interpretations appeared to permit different responses to red and blue light in relation to mesocotyl elongation, depending upon the nature and distribution of the absorbing pigments. If mesocotyl elongation was slowed down by red light one might anticipate an early chlorophyll involvement, in contrast to the delayed involvement of chlorophyll in the development of the corn seedling. Yet there was also the indication that, even with the type of pigment distribution present in the corn used in these experiments, the temperature had an important relation to the time at which mesocotyl elongation ceased irrespective of light conditions, and hence an important relation to the duration of the exposure to light. For an adequate detection or demonstration of the potential effectiveness of light, a low growth temperature was indicated, particularly with plants of a moderate optimum temperature and a modest mesocotyl extensibility, such as that of oats.

The results appeared to offer further evidence of an association between carotenoid pigments and a restraining action on cell elongation, such as that involved in phototropism.

Summary

A series of experiments with corn seedlings gave results interpreted as evidence that up to the time of the emergence of the leaf-blade from the coleoptile, when mesocotyl elongation ceased irrespective of light conditions, the elongation of the mesocotyl was retarded by blue light, and unaffected by red light.

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CRYOSCOPY OF SMALL AMOUNTS OF EXPRESSED TISSUE SAP

H. B. Currier

(WITH FOUR FIGURES)

In the course of an investigation dealing with the water relations of root cells of red beet (*Beta vulgaris*), it was necessary to devise a method whereby osmotic pressure values of 0.5 to 1 ml. of sap, expressed from small blocks of tissue, could be obtained. The cryoscopic apparatus and procedure described in this paper are simple, and permit up to 6 determinations in an hour.

Sap expression

Tissue cylinders 2.8 cm. long and 0.9 cm. in diameter were removed from roots by means of a cork borer and placed in small vials just large enough to accommodate them. A stopper was inserted, sealed with paraffin, and the vial placed in a freezing environment. The temperature required for rapid and complete freezing is dependent on the kind of tissue. For beet root, $-15°$ C. was found sufficient; for other plants, conifer needles for example (**5**), the temperatures of liquid air or solid carbon dioxide are necessary.

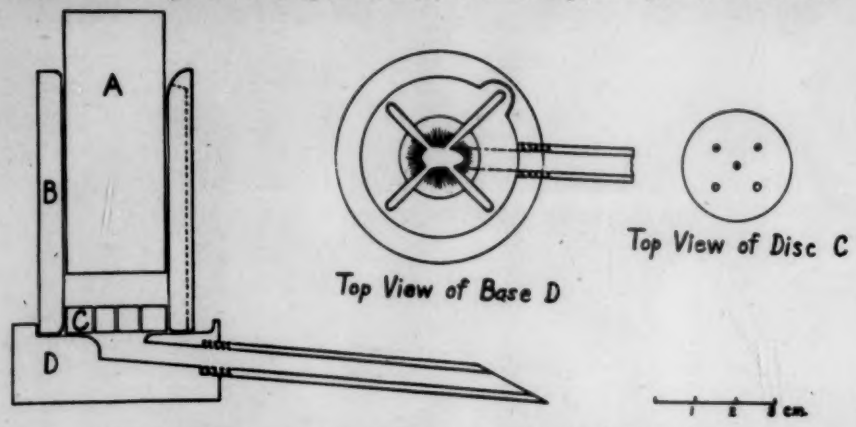
Expression of sap was accomplished by the use of the small press cylinder shown in figure 1, in connection with a hydraulic press equipped with a gauge calibrated in pounds per square inch. The press cylinder outfit was precision made and was, with some modification and reduction in size, patterned after that designed by Broyer and Furnstal (**1**). It was composed of Monel metal with exception of the piston (A), which was of stainless steel. The cylinder (B) had an inside diameter of 1 inch.

In pressing a tissue sample, a pad of 8 thicknesses of cheesecloth was placed on top of disc (C). The frozen tissue was removed from the small vial, wrapped in an 11×11 cm. square of cheesecloth, and quickly inserted into the press cylinder. Pressure was slowly increased to 10,000 to 11,000 pounds per square inch. This was shown on the pressure gauge as 2840 to 3120 pounds per square inch, since the ratio of end surface of the press cylinder piston and the hydraulic piston was $\frac{17.8 \text{ sq. cm.}}{5.05 \text{ sq. cm.}} = 3.53$. Collection of the approximately 1 ml. of sap consumed 3 to 4 minutes. The time involved and the heat capacity of the metal were such that the tissue was completely thawed by the time pressure was applied.

Freezing point determination

The cryoscopic apparatus is illustrated in figure 2. The container is a half-gallon thermos jug, with glass wool insulation (H) packed between an external metal covering and an inner vessel of earthenware. Replacing the lid and screw cap is a top (D) composed of Masonite, with holes drilled to receive a heavy wire stirrer (A), an ordinary thermometer (B) reading to

−20° C. to indicate bath temperature, a glass air jacket (K), and a small glass tube (J) in which a water-filled glass capillary (G) can be frozen. Within the air jacket a sample tube (I) is held in place by a cork fitted with



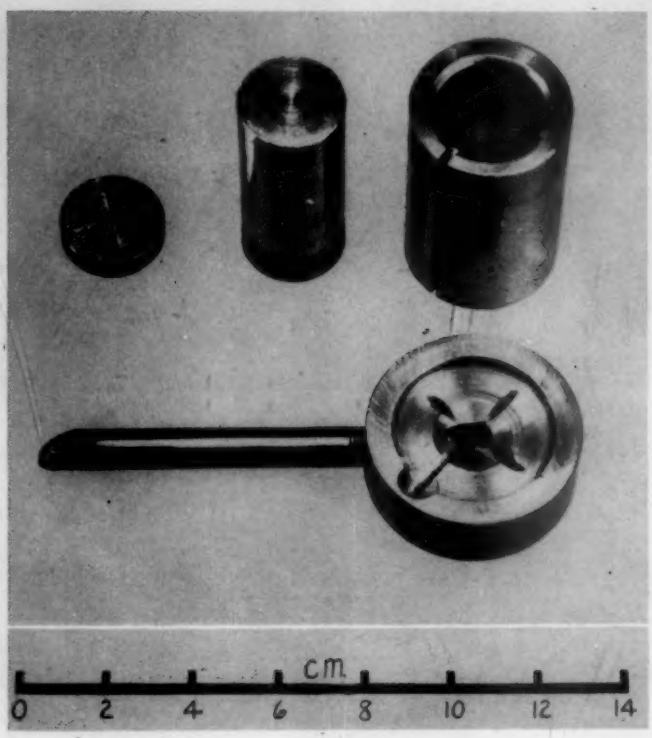


FIG. 1. Press cylinder outfit used to express small amounts of sap from plant tissue. Part 1, diagram; part 2, photograph of parts.

holes to accommodate the freezing point thermometer (C), a fine wire stirrer (E) tipped with rubber, and a small hole (F) through which the glass capillary could be inserted into the test solution.

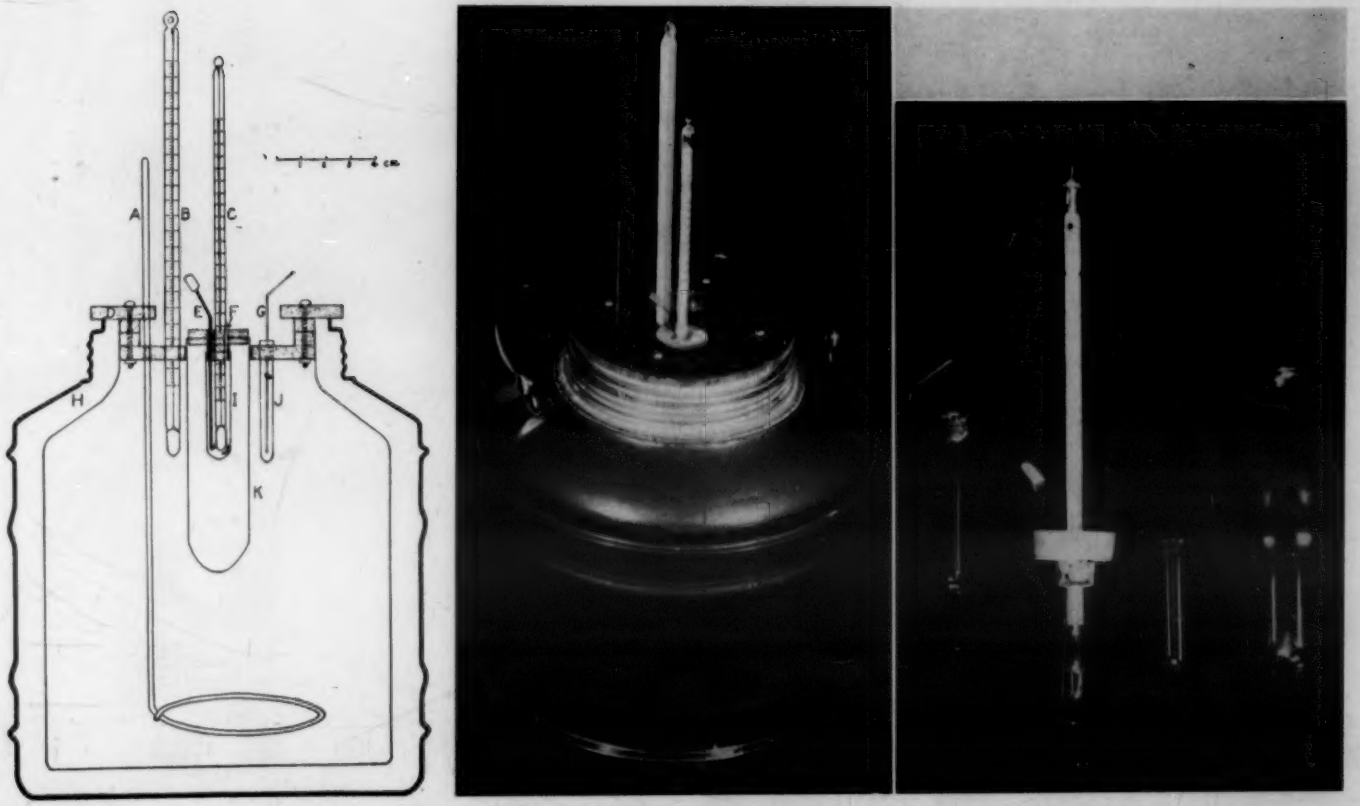


FIG. 2. Construction of cryoscopic apparatus. Explanation in text. Part 1, diagram; part 2, photograph of assembled apparatus; part 3, thermometer, tubes, etc., used in the freezing of sap.

The freezing point thermometer was specially made, and obtained from Braun-Knecht-Heimann Co., San Francisco, Calif. Specifications called for an instrument of the Anchutz type, reading from +1 to −3.5° C., calibrated in fiftieths of a degree, and having a very small bulb (0.5 cm. in diameter and 1.0 cm. in length).

For the determination, the container is filled with an ice-salt mixture at approximately −10° C. A dropping pipette serves to introduce 0.7 ml. of sap into the sample tube, after which the small thermometer is placed in position so that the bulb is completely immersed. The sample tube is then placed in an ice-water bath in a smaller double-walled vessel until the thermometer registers about +0.5° C. Then, after excess water has been carefully removed, it is transferred to the air jacket of the cryoscope. This operation saves considerable time when a large number of determinations are being made. As the mercury nears −2° C. stirring of the solution is begun with an up and down motion of the left hand, repeated about 3 times per second. When the thermometer reads −2° C., crystallization is induced by momentarily inserting the tip of the ice-filled glass capillary into the solution through the small hole (F). The mercury immediately rises to a maximum and remains for a minute or so, then drops slowly. Stirring is continued until the plateau in the freezing curve is reached. The highest temperature is recorded as the observed depression ($\Delta_{obs.}$). A small magnifier aids in reading the thermometer.

Standardization of apparatus

Because readings of any cryoscopic apparatus of the type described above are subject to errors caused by (1) supercooling of the solution, and (2)

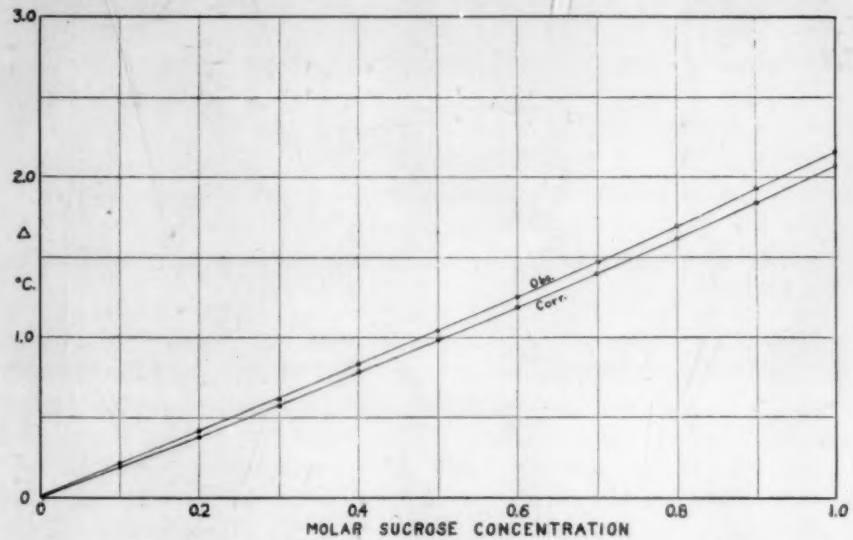


FIG. 3. Freezing point depression (Δ) of sucrose solutions. The two lines indicate differences between observed (obs.) and correct (corr.) values.

absorption of heat by the system during freezing, the equipment must be standardized. For this purpose weight molar sucrose solutions of various concentrations were prepared, using reagent grade sugar and water which had been previously boiled to remove air.

The observed freezing points were compared with accepted cryoscopically determined values found in the International Critical Tables (**3**) by means of the graph shown as figure 3. The discrepancies varied from 0.02° C. for a 0.1 molar to 0.11° C. for a 1.0 molar solution. By referring the observed Δ to this graph the corrected Δ could be read off directly to 0.01°.

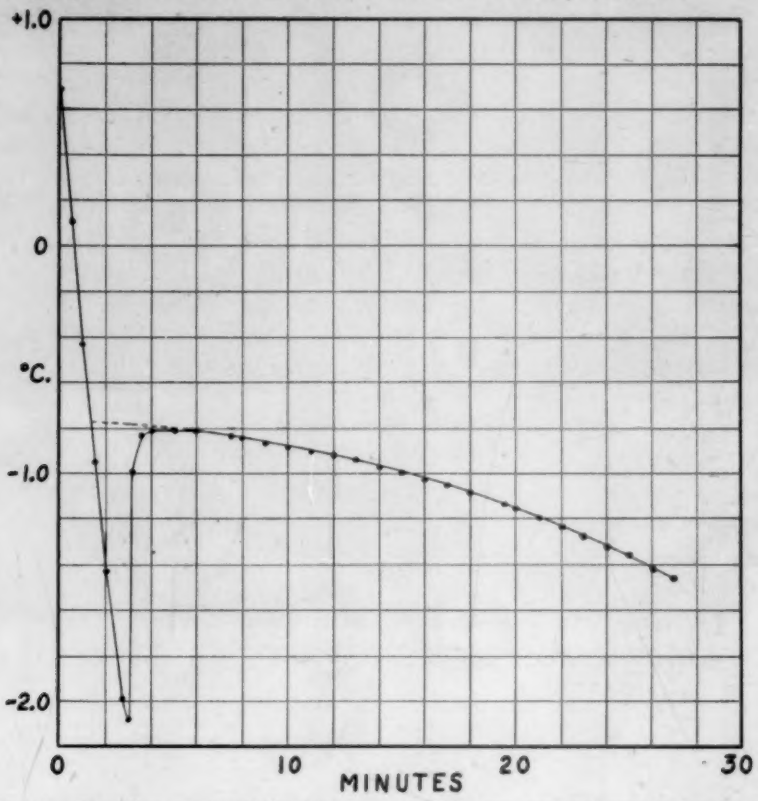


FIG. 4. Freezing curve obtained by means of the apparatus shown in figure 2, using 0.7 ml. of beetroot sap.

Although the discrepancies between the true and observed values seem large, the extremely consistent results proved the accuracy of the measurements. A suitable and convenient correction for the effect of supercooling in giving Δ values which are too great has been suggested by HARRIS and GORTNER (**2**), and is commonly used. An approximate correction for heat capacity effects can also be calculated if, (1) the weight and heat capacity of glass and stirrer in contact with the solution, (2) volume of solution, (3) temperature at which freezing begins, (4) temperature of bath, and (5) observed freezing point, are known.

In the present procedure separate corrections for these errors are unnecessary, since by accurately calibrating the apparatus and using exactly the same technique each time, one needs only to refer the observed value to the graph to obtain the corrected value. The increasing discrepancy between $\Delta_{obs.}$ and $\Delta_{corr.}$, as the solution becomes more concentrated, is not due to supercooling effects, as this correction, when calculated by HARRIS and GORTNER's formula, remains at about 0.01° C. for all concentrations (about 0.005° for 0.1 M). The probable explanation is that with increasingly concentrated solutions a greater amount of heat is lost to the system, due to slower crystallization and the longer time required to reach the apparent freezing point. Against WALTER's (**6**) recommendation that the amount of supercooling be always 1.5° C., there is a distinct advantage in initiating the freezing at a definite point, since with an unknown solution no preliminary trial is necessary in order to determine where freezing must begin to be exactly 1.5° below the freezing point.

As proof of the validity of correcting observed freezing point values as described, freezing curves, such as that shown in figure 4, were constructed as recommended by MAIR, GLASGOW and ROSSINI (**4**). Extrapolation of the part of the curve where thermodynamic equilibrium exists between the solid and liquid phases of the system, back to the initial downward part of the curve, gives a close approximation of the true freezing point. These authors point out that in a strict sense the true freezing point is slightly higher than this, but have mathematically proved that the difference is insignificant when the amount of undercooling is small in comparison with the head of temperature (difference between temperature of the freezing bath and that of the test solution). With a head of temperature of 10° to 15° C. this error was found to be unimportant in the determinations made by the method described above. True freezing points of both sucrose solutions and beet juice obtained by plotting freezing curves agree with those corrected by means of the calibration curve (fig. 3).

While the method of calibration employed is probably applicable to all plant juices approximating beet sap in composition and freezing behavior, it may not be suitable for highly colloidal solutions, where ice formation occurs more slowly. Where doubt exists freezing curves will indicate whether or not the method can be used.

This work was carried out while the writer was associated with the Division of Botany, University of California, Davis, California. Grateful acknowledgement is made to Dr. A. S. CRAFTS for helpful suggestions, and to Mrs. DOROTHY HARTMANN for preparing the drawings.

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IS NATURAL GAS INJURIOUS TO FLOWERING PLANTS?[1]

FELIX G. GUSTAFSON

(WITH TWO FIGURES)

It is a well-known fact that manufactured illuminating gases are injurious to many flowering plants (**1, 2, 3, 4, 5, 6, 8, 9**). With the extensive use of natural gas it has become increasingly important to know what is its effect upon plants. SOLHEIM and AMES (**7**) seem to be the only ones to have investigated this. These investigators found that tomato, potato, sunflower, castor bean and geranium plants were not injured by concentrations as high as 50 per cent. of natural gas in air over a period of 72 hours. Cut carnations were not injured by a concentration of 2 per cent. during a 96-hour exposure. Fuchsia plants had the leaves browned very slightly during a 96-hour exposure to a 50 per cent. concentration of natural gas in air. These tests were made in Wyoming and the source of the gas is unknown.

Many cities in the south-eastern part of the state of Michigan are now using natural gas obtained from the Texas fields. It has, therefore, been considered worthwhile to locally investigate its effect upon some common greenhouse and home plants.

Procedure and material

The plants were exposed to the natural gas in air mixture under bell jars. The bell jars varied in size from 3 to 5 gallons. Each bell jar was set in a metal pan partly filled with water (fig. 1), thus making a water seal. The pots with the plants were set in cups made from tin cans to prevent the soil from becoming water saturated. To note the rate of gas flow the outlet tube dipped into water in a washing bottle, and the rate of bubbling was noted. The gas was led outdoors after passing through the bell jars to avoid the contamination of the atmosphere in the greenhouse. In some experiments 3 experimental bell jars were used for 1 control jar, while in others an equal number of experimental and control jars was used.

The gas was taken from the Ann Arbor, Michigan, gas mains and piped into a small greenhouse compartment, where the experiment was conducted. The gas was mixed with compressed air in a gas mixing chamber, and piped from there into the bell jars. For the controls the compressed air was led directly into the bell jars. The gas and air flow were separately measured by gas meters, or orifice flow meters. The differential pressure across the orifices was continuously indicated by differential angle manometers (fig. 2). The atmosphere in the bell jars was also frequently checked for gas content with a calibrated combustible gas detector. The usual gas mixture flow was about two cubic feet per minute, but part of the gas mixture was led out-

[1] Paper from the Department of Botany of the University of Michigan, no. 737, in cooperation with the Michigan Consolidated Gas Company.

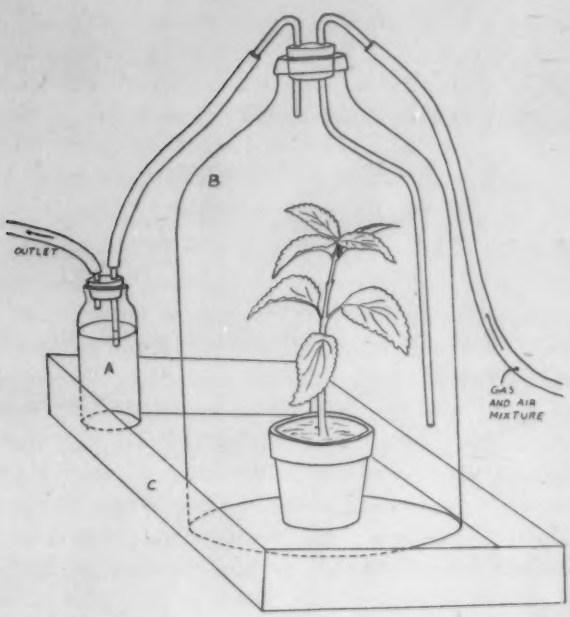

FIG. 1. Set-up of the gas chamber. Bell jar, B, is set in a metal tray, C, partly filled with water to give a water seal. The pot with the plant is set in a metal cup (not shown) to prevent excessive saturation of the soil. The air leaving the bell jar is passed through a bubbler, A, to note the rate of gas-air flow.

doors through a by-pass. It was found that a too rapid flow through the bell jars had a drying effect upon the plants.

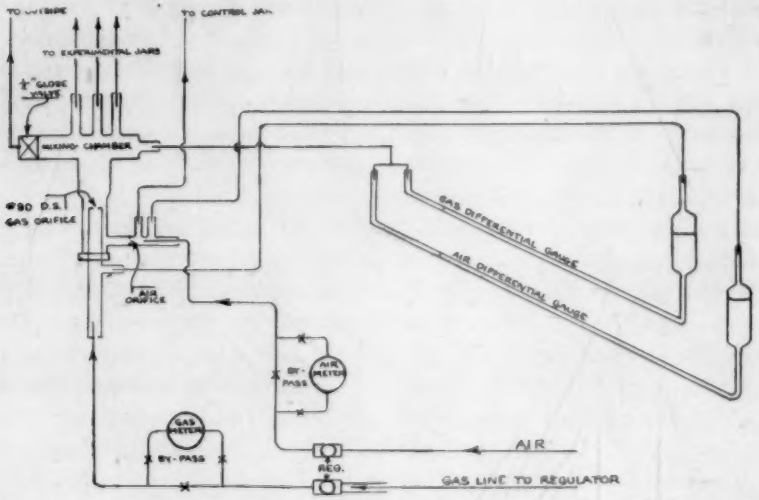

FIG. 2. Arrangement of the gas and air lines, the mixing chamber, the outlet tubes and the differential angle manometers, by means of which the rate of gas and air flow was observed without the use of the meters. The meters were usually disconnected except once or twice a day when the flow was checked.

For the most part the plant material consisted of potted plants, but cut flowers and branches were also used. In the latter instances, the cut stems were placed in water in bottles. If the experiment lasted several weeks the water was changed weekly and the stem ends cut off a centimeter or two each time.

Investigations have shown that some plants are much more sensitive to manufactured gas than are others. From lists of sensitive plants compiled by different investigators as many different kinds as were available were selected. The potted plants used were: tomato, yellow green Coleus, sunflower, snapdragon, Cyclamen, marigold (several varieties), *Kalanchoe tubiflora* and sensitive plant. Cut flowers in bud stage, of tulip, carnation and stock were used and also cut branches of Swainsonia and Bougainvillea. Germination of radish, wheat, sunflower, squash, and lupine seeds was investigated.

Results

In each experiment several species of plants were used, but in presenting the results one plant at a time will be considered, with all the information gained. It is felt this method of presentation will be preferable to a discussion of the data, experiment by experiment. After all, if one is interested in a particular plant it is preferable to find all of the information in one place rather than scattered throughout the entire investigation.

Tomato

The epinastic response of the tomato to manufactured gas is well known and very easy to observe. The plant is very sensitive, therefore it was used in six of the fifteen experiments run in this investigation.

In these experiments the natural gas concentration in air varied from one per cent. to two per cent. and the experiments lasted from 9 to 21 days. The plants were not injured and showed no epinasty in any of the experiments with natural gas. In two experiments where a small percentage of manufactured gas had been introduced for a few hours, some injury was observed. In these two experiments the plants showed temporary epinasty. As they were, however, exposed for only a short time to the mixture of natural and manufactured gases, they recovered without permanent injury.

At certain times during the experiments small percentages of manufactured gas were introduced into the test gas. The plants revived within a few hours after the manufactured gas was removed from the test gas. This indicates that the plants used and the experimental set-up was such that if natural gas had been injurious, the plants would have shown epinasty and other injury very quickly.

In one experiment the growth of the plants in length was measured, besides noting the general response. The result of this experiment was that three plants outside of the jars grew 8.9 cm., one plant in air under a jar grew 7.3 cm. and four plants in natural gas under the bell jars grew 8.8 cm. Certainly the plants in gas did not grow any less than those in air. One

can say definitely from these experiments with young tomato plants that they are not injured by natural gas in air in concentrations as high as 2 per cent., during a period of 21 days.

Yellow green Coleus

Four Coleus plants were exposed for 14 days to one per cent. of natural gas in air without any sign of injury. After 3 days in air they were further exposed to gas for a period of 19 days. During this period they were exposed to some manufactured gas. At the end of the second period two plants showed a slight epinasty. The natural gas when uncontaminated with manufactured gas was not injurious to Coleus.

Sunflower

Five small sunflower plants were exposed to one per cent. of natural gas in air for a period of 13 days. There was no injury sustained by these plants. Their growth in length was also measured, and it was found that the average growth was 4.6 cm. compared with 3.6 cm. for two similar plants outside of the jars and 3.5 cm. for one plant in air under a bell jar. The plants in the gas mixture under the bell jars grew a little more than those in air.

Snapdragon

The experiment in which this plant was used was run during the period when occasionally manufactured gas was introduced. One plant was under the jar with air and three under jars with one per cent. gas in air mixture. After 12 days exposure to the gas-air mixture the plants were uninjured.

Cyclamen

Small Cyclamen plants were used in two experiments: two plants in air, and 9 in gas-air mixtures. In the first experiment a one per cent. natural gas in air mixture was used for 14 days. In the second experiment lasting 21 days, gas mixtures from 1.7 to 2.1 per cent. were used. At the end of both experiments the plants were in perfect condition. Some leaves had died and new ones had been produced, but as this had also happened to the control plants it is considered only a natural process of growth. These plants were kept under observation for a number of weeks after they were removed from the bell jars and no difference in development was observed in those plants that had been in air and those in the gas mixture.

Kalanchoe tubiflora

Kalanchoe plants 4 to 5 inches tall were used in one experiment. One plant was in air and three in natural gas-air mixture ranging from 1.7 to 2.1 per cent. The experiment lasted 21 days. There was no difference in appearance of any of the plants at the end of the test. After the experiment the plants were kept for several weeks and all grew normally.

Sensitive plant

Eight sensitive plants about 7 inches tall were used in one experiment lasting 16 days; 2 were in air and 6 in two per cent. natural gas-air mixture. On the tenth day of the experiment the plants under 2 of the bell jars with the gas mixture had a few of the lower leaves become yellow and drop off. The soil in which these plants grew was quite dry. As these plants lost no more leaves after the soil was watered the loss was undoubtedly due to drought. At the end of the experiment all plants were in good condition. They remained under observation for a number of weeks afterward and showed no indication of injury.

Marigold

Sixteen marigold plants were used in two experiments; 4 in air and 12 in natural gas-air mixture. The first experiment lasted 21 days and the gas-air mixture varied between 1.7 and 2.1 per cent. The second experiment also lasted 21 days and the gas in air mixture varied from 1.8 to 2.5 per cent. At the end of both experiments there was no observable difference between the plants in air and those in the gas mixture. These plants were also kept under observation for some time after the conclusion of the experiment and they all developed normally.

Tulip

Tulip flowers are easily disfigured if the developing plants and buds are exposed to manufactured gas. It was therefore very desirable to expose the developing buds of these plants to a mixture of natural gas in air. Unfortunately it was not possible to use intact plants as no experiments were run during the time dwarf tulips were on the market. The plants used had to be cut in order to get them under the bell jars. Eighteen plants with buds were obtained, and 5 were placed under a jar with air and 13 under jars containing natural gas-air mixture of one per cent. The experiment lasted 7 days. During this time the stems elongated considerably in all plants. The petals dropped off of some plants, not off of others, but there was no difference between those exposed to gas and those exposed to air. There was no deformation of the petals of any of the flowers. Evidently natural gas does not injure tulip flowers.

Carnation

The carnation is another cut flower which is easily injured by manufactured gas. Twenty carnation stems each with several buds in different stages of development were obtained, and 5 were placed under jars with air, and 15 under jars with natural gas-air mixtures varying from 1.7 to 2.1 per cent. The experiment lasted from 14 to 17 days. The water in which the plants were placed was renewed several times during the experiment, and the stems were cut each time. The high humidity was a favorable

medium for fungus growth and some badly infected plants were removed from the jars 14 days after the start of the experiment. The others were put back for 3 more days. There was no appreciable difference in the time of opening of the buds in the different jars, but what slight difference there was, was in favor of those in the gas mixture. The fungus infection was equally bad in all jars. The buds all opened very well, forming perfectly normal flowers.

Stock

Cut flowering stems of this plant were used in one experiment; 2 in air, and 6 in one per cent. natural gas-air mixture. The length of exposure was 13 days. The buds opened normally and the flowers remained in good condition for the same length of time on the plants in gas as in air. At the end of the experiment there was no difference in the appearance of the plants.

Bougainvillea and Swainsonia

Only large plants were available and fresh cuttings were therefore used. Swainsonia is one of the plants most sensitive to manufactured gases. Leaf-

TABLE I

Percentage germination of lupine, radish, wheat, squash, and sunflower seeds when subjected to atmospheres varying from 1.5 to 5.0 per cent. natural gas in air, to 100 per cent. air under bell jars, and outside of the confinement of bell jars. Figures within parentheses denote the number of seeds used

Treatment	Lupine	Radish	Wheat	Squash	Sunflower
In air	67.5 (40)	50.0 (90)	90.0 (90)	41.2 (34)	16.6 (60)
In gas	95.0 (40)	77.0 (90)	93.3 (90)	58.8 (34)	16.6 (60)
Outside of the jars	100.0 (40)	90.0 (150)	93.3 (45)	30.0 (17)	23.3 (30)

lets drop off in very short order, when the plants are exposed to manufactured gases. The two plants were used in the same experiments and are therefore reported together. They were employed in 4 experiments. Both Bougainvillea and Swainsonia were injured by natural gas in air when concentrations were as high as 2.0 per cent. and the time of exposure was 14 days. If there was an injury caused by a 1.0 per cent. natural gas in air mixture during an exposure of two weeks it was very slight. The two plants lost their leaves or leaflets as a result of exposure to the 2 per cent. mixture of natural gas in air, but the leaves which dropped differed in the two plants. In Swainsonia the older leaves dropped, but the young growing tips were never injured even with natural gas concentrations as high as 3.0 per cent. and an exposure time of 4 weeks. The Bougainvillea on the other hand, lost its younger leaves first and the old leaves were lost only after long exposures.

Seed germination

Seeds of radish, wheat, sunflower, squash, and lupine were used in several experiments on germination. The seeds were planted in pots in the

usual way and placed under the bell jars. The first experiments were made during the winter when the greenhouse was fairly cold near the windows and the results were questionable, because some of the experimental jars were near the windows, and all the control jars were toward the inside of the room where the temperature was higher. In studies made during late summer the results were clear cut as shown in table I.

The evidence is clear that the natural gas mixture does not reduce the percentage of germination of these five seeds.

Discussion

The injurious action of manufactured gas has been shown to be due to ethylene and carbon monoxide. The natural gas used in these experiments is composed of 74.6 per cent. methane, 14.2 per cent. ethane, 10.9 per cent. nitrogen, 0.2 per cent. oxygen and 0.1 per cent. carbon dioxide. As this gas contains neither ethylene nor carbon monoxide it is easy to see why it should be non-injurious to plants. Concentrations of natural gas in air as high as one per cent. were non-injurious to all plants tried and higher concentrations were injurious only to Bougainvillea and Swainsonia. If these results are translated into situations found in the home or commercial greenhouses we find that there never should be any injury from natural gas to the plants grown in them. Concentrations of gas in homes or greenhouses are so low that it has been impossible to determine them by accurate chemical methods.

Summary

It has been shown that natural gas in air is not injurious to yellow green Coleus, sunflower, snapdragon, stock, and tulip plants in concentrations of one per cent.; higher concentrations were not used with these plants. Tomato, Cyclamen, marigold, sensitive plants, *Kalanchoe tubiflora*, and carnations were not injured by concentrations of two per cent.; higher concentrations were not used. Bougainvillea and Swainsonia were not injured by one per cent. natural gas in air, but they were injured by concentrations of two per cent. Seeds of lupine, radish, wheat, squash, and sunflower germinated as well in 1.5 to 5.0 per cent. natural gas as in air.

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BRIEF PAPERS

ISOLATION IN UNIALGAL CULTURE OF LICHEN GONIDIA BY A SIMPLE PLASMOLYSIS TECHNIQUE

E. R. Bogusch

The separation of gonidia from the lichen fungus and growing them in unialgal cultures has been difficult in routine studies of lichen algae. The difficulty has been largely one of completely excluding surface organisms clinging to the lichens. This is especially true of epiphytic unicellular algae or other casual forms temporarily upon the lichen. Such unwanted species in a culture of gonidial algae sometimes confuses the identity of the latter.

No difficulty is associated with the growth of the gonidial algae themselves when they are placed in a suitable culture medium. Chodat (**1**) and Geitler (**3, 4**) prepared cultures by very simple techniques. The manner in which Chodat prepared his gonidial cultures failed to assure *per se* unialgal development and the algae needed careful separation to effect isolation of the desired forms. Geitler's simple inoculation of thin sections of lichen thalli into culture media is open to the same objections.

The application of Skinner's method (**7**) to the separation of gonidial cultures is helpful but time consuming. Gonidial algae require weeks to grow and additional steps delay investigations which require a certain stage of maturity for study.

Jaag's use of the micromanipulator (**5**) is excellent and has the outstanding value of exactness. It is, however, not always within the reach of research students and is seldom available in the average laboratory. The use of glass micropipettes by the author has shown similar results, but this method requires the development of skill.

During the course of several years of study devoted to the gonidial algae of *Parmelia perforata* and *Ramalina complanata* the need for a rapid, sure technique was obvious. Czurda (**2**) succeeded in freeing filamentous algae from other species by repeatedly washing the specimen with sterile water. Geitler (**3**) used a similar method with pieces of lichen thallus, apparently with good results.

The author found, however, that even with repeated washings, pieces of the thallus of both Parmelia and Ramalina often introduced other algae into the cultures. Some of these undesirable forms could be eliminated through careful selection of healthy pieces of lichens and trimming away all dead parts before preparing them for inoculation. Enough contaminated cultures still appeared to prompt a more careful study of techniques to facilitate subsequent researches.

A large number of culture media intended for use in growing unicellular algae have appeared in literature. As far as these have been examined, they all appear excellent when applied to the purpose for which

they have been intended, but vary when used for gonidial cultures. Most glucose media have proved unsuccessful for the primary separation cultures, because the use of glucose encourages the rapid growth of molds, which are difficult to keep out, and obscure the results. Some molds are definitely detrimental to the gonidial algae themselves.

GEITLER (**3**) employed soil extract agar and a 0.05 per cent. Knop solution with success. His ability to eliminate many undesirable organisms from his cultures is no doubt partly due to his choice of media. Molds and bacteria fail to grow well upon either of these substances. In the author's laboratory it was demonstrated that both soil extract agar, and agar medium containing Knop solution, were superior to others for the preparation of the initial cultures. Some exceptional cultures were maintained without transfer for as much as two years. The method of preparing soil extract media given by JOHANSEN (**6**) was successfully used with little modification and seems to possess advantages over Knop agar in that colonies are often more vigorous. Of the several dilutions suggested by JOHANSEN, all worked effectively, apparently demonstrating that within the limits of the concentrations suggested, the details of the preparation of the medium introduce no critical factors.

To clean the lichen thallus of surface organsims requires a process not injurious to the cells of the gonidial layer. Thus a solution of low toxicity and relatively poor penetration powers is required. A 10- to 30-second immersion of the thallus in 50 per cent. alcohol, followed by rapid washing gave inconsistent results. Some excellent cultures of gonidial algae free from contamination were obtained in this manner. In other preparations the gonidial algae were likewise destroyed or seriously injured. The amount of moisture in the thallus seemed to influence the degree of injury.

Plasmolysis of surface organisms by means of 2 M solutions of potassium chloride or magnesium sulphate has given the best results of any methods tried so far. The technique is simple and rapid. Pieces of the lichen are selected from healthy material and washed in one or more changes of sterile water until no soil or foreign material remains attached. The pieces of the lichen are then placed in a test tube or vial and rapidly shaken with the strong saline solution for about fifteen seconds, after which the contents are quickly rinsed with sterile water for five or six changes of water.

If glucose media are employed for primary cultures, bacteriological cleanliness must be observed at every step to avoid molds. By using soil extract media the cultures may be initiated by dropping a whole piece of the prepared lichen into the medium. A more desirable way to produce rapid response is to grind the thallus lightly in a mortar with powdered glass or to crush it on a clean microscope slide with a scalpel so that the gonidia may be partly freed from the fungus.

Experiments have indicated that cultures initiated in liquid media make a more rapid immediate development than do those started upon solid media. By careful study of over six hundred cultures made by the technique de-

scribed, none has shown any algae other than those belonging to the gonidial layer. The gonidial algae develop in liquid media in about four to six weeks when kept in diffuse light and at a temperature under 27° C. The algae may then be transferred by platinum loop or pipette upon special media for further study. Gonidial algae grown in unialgal culture in this manner have been observed throughout their complete life cycle.

Summary

1. The algae from the gonidial layer of certain lichens have been found easy to grow upon artificial media.

2. Soil extract media and Knop solution were demonstrated superior to those containing glucose for primary cultures by reducing the conditions favorable for the growth of molds.

3. Plasmolysis of organisms clinging to the surface of the lichen by means of strong solutions of potassium chloride or magnesium sulphate was shown to provide unialgal cultures of lichen gonidia with a high degree of certainty.

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USE OF MONOCHLORACETIC ACID TO INCLUDE AMMONIA IN THE "SOLUBLE NITROGEN" TISSUE TEST[1]

E. M. EMMERT

In a tissue test previously reported,[2] it was found that while amino nitrogen was oxidized to nitric acid, amide and ammonia nitrogen were not. While ammonia nitrogen in tissue extracts is not large, considerable amide nitrogen probably is present.

Since it was shown[2] that amino nitrogen is readily oxidized in the test, a means of converting ammonia nitrogen into amino nitrogen would make it include both amide and ammonia nitrogen. Amide nitrogen readily hydrolyzes to ammonia. By treating with strong alkali in the presence of a large amount of monochloracetic acid, it was found that the ammonia was taken up to form glycine which would be oxidized to form nitric acid, in the test.

Procedure

Place 1 gm. of monochloracetic acid in a 30-ml. test-tube. Add 0.5 ml. of plant extract, adjusted so that it does not produce too heavy or too light a color to compare with a standard containing 0.000625 mg. of N per ml., when the nitrate color is developed. Add 1.2 ml. of 40 per cent. NaOH with shaking, and 0.5 ml. of 40 per cent. $NaClO_4$ solution. Add 2.5 ml. of fuming sulphuric acid (15 per cent. SO_3) just slowly enough to prevent too violent boiling. Blow most of the chlorine fumes out with a bent glass tube. A yellow chlorine color should persist at first but gradually fade. Not all of the color has to fade out. Dilute with 50 per cent. H_2SO_4 to 10 ml. (more or less, according to the content of nitrogen in the sample. If nitrogen is low, no dilution should be made, but the entire mixture should be treated with phenoldisulphonic acid). Place 1 ml. in a test-tube and add 3 ml. of phenoldisulphonic acid. Mix well and add 10 to 15 ml. of water. Neutralize with 40 per cent. NaOH, adding a small excess over what it takes to bring the yellow color, cool and make up to a convenient volume. Compare with a standard containing 0.000625 mg. N per ml. This concentration of standard reads best in a photoelectric colorimeter since readings on light-colored solutions are most accurate. The standards and blanks should be carried through the same procedure as the unknown.

Results

Table I shows results on pure ammonia solutions.

This shows proportionate recovery of all the ammonia added. When 0.01 mg. of ammonia was added to each of two tissue extracts, the recovery was 0.0109 mg. in one and 0.0110 in the other.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] EMMERT, E. M. Determination of ammonia and amide nitrogen in connection with the chlorate method for nitrogen in plant tissues. Plant Physiol. 14: 341–349. 1939.

TABLE I

NITROGEN IN PURE AMMONIA SOLUTIONS

	NH_3 ADDED AS NH_4Cl	FOUND
	mg.	*mg.*
Pure solution	0.500	0.504
" "	0.250	0.252
" "	0.125	0.126

Table II shows that considerably more nitrogen is found when monochloracetic acid is used in testing plant extracts.

TABLE II

EFFECT OF MONOCHLORACETIC ACID

NO MONOCHLORACETIC	1 GM. MONOCHLORACETIC	RATIO
p.p.m.	*p.p.m.*	
1760	3264	1.85
680	1280	1.88
960	1632	1.70
960	1680	1.75
1000	1680	1.68

It will be noted that the ratio of increase is nearly the same in all of the extracts. This does not give absolute proof that total nitrogen was determined in the extract, but it is pretty certain that all nitrogen goes over to nitrate nitrogen when monochloracetic acid is used. Further work is planned to test this for total nitrogen in plant tissue.

THE KENTUCKY AGRICULTURAL EXPERIMENT STATION
LEXINGTON, KENTUCKY

NOTES

Annual Election.—The twenty-first annual election of the American Society of Plant Physiologists has resulted in the election of the following officers for 1944–1945:

President, Dr. H. A. SPOEHR, The Carnegie Institution of Washington, Stanford University, California.

Vice-President, Dr. F. W. WENT, The California Institute of Technology, Pasadena, California.

Elective member of the editorial board, Dr. D. R. HOAGLAND, Division of Plant Nutrition, The University of California.

As the meeting for 1944 is so near at hand, the new officers have short time to meet the exigencies of the situation. It will be a great aid if members will cooperate fully with the officers in bringing all plans to an early completion. Fortunately, the program committee is busy with the details and it should not be too difficult to complete the tasks promptly.

With this year the Society reaches its majority. Twenty-one years of testing experience has given us strength, poise, confidence, and judgment. We are ready to play our full part in the unfolding drama of science in a free world.

Cleveland Meeting.—If you are planning to attend the only meeting of the American Society of Plant Physiologists for 1944 to be held at Cleveland in September, make your reservations early. A central agency has been established for handling requests for room accommodations. Make your requests for room to the Cleveland Convention Bureau, A.A.A.S. Meeting, 1604 Terminal Bldg., Cleveland 13, Ohio. They ask that you name three hotels of your choice, the price of room desired, time of arrival, and time of departure from Cleveland. Please also plan to share your room with some friend or neighbor. You are asked to forego the privilege of single occupancy in the interests of accommodations for all. Details will be published in *Science* and in the *A.A.A.S. Bulletin*. Keep on the alert for facts about the meetings; your full cooperation will be appreciated, and will pay dividends in comfort. Send in your request to the central agency as soon as you are certain of your plans.

The principal hotels are the Statler, Carter, Cleveland, and Hollenden. Rates announced are as follows:

Statler: Single, \$3.00—\$6.00; double, \$5.00—\$8.00.

Carter: Single, \$3.00—\$6.00; double, \$4.50—\$9.00 (twin bedded).

Cleveland: Single, \$3.00—\$7.00; double, \$4.50—\$9.00, and \$6.00—\$12.00 (twin bedded).

Hollenden: Single, \$3.00—\$5.00; double, \$4.50—\$6.50 and \$5.00—\$12.00 (twin bedded).

Life Membership Committee.—The life membership committee to select the recipient of the 22nd award of the CHARLES REID BARNES life membership in the Society has been named by the president, Dr. H. A. SPOEHR. The chairman of the committee is Dr. F. P. CULLINAN, the U.S.D.A., Washington and Beltsville, Md., and his colleagues on the committee are Drs. R. B. WITHROW, Purdue; P. R. GAST, Harvard; J. BONNER, California Institute of Technology; and WM. SEIFRIZ, The University of Pennsylvania. It is hoped that the selection can be made in time to announce the award at Cleveland in September.

Cleveland Program.—An excellent program is in preparation for the Cleveland meeting of our Society. The details are still not ready for publication, but will be printed and sent out by the Secretary as soon as possible. Many interesting topics will come up for discussion. In the business meetings it would no doubt be desirable to discuss the possibilities of closer relation of the work of plant physiologists to war conditions. The editor is of the opinion that everything we do during times of peace is of direct value in time of war, and that we should not be expected to be able to eliminate the time-factor during a war. Even if we had all the knowledge requisite to a rubber production program, for instance, it would require several years to provide the acreage necessary, unless time had been taken by the fore-lock. What should plant physiologists do in times of national crisis? Round table discussions of this topic would serve to clarify our vision.

Postponement.—Owing to conditions beyond our control it has not been possible to include the biography and portrait of Dr. TOTTINGHAM in this issue of PLANT PHYSIOLOGY. We still hope that we shall be able at no far distant date to publish an account of his life as a memorial to him and his long and eminent service to plant biochemistry and plant physiology.

Sir Edward John Russell.—It is a great pleasure to present the biography and portrait of Sir EDWARD JOHN RUSSELL, who has recently retired from the directorship of the Rothamsted Experimental Station.

Sir JOHN is a corresponding member of the American Society of Plant Physiologists. We take this occasion to express to him our admiration and our keen sense of appreciation of his long service to plant science, and to wish him many more years of happy activity. It is a great pleasure to dedicate this number of PLANT PHYSIOLOGY to him in honor of the approaching seventy-second anniversary of his birth. Additional portraits have been made, and are available along with complete sets of those published in past years in PLANT PHYSIOLOGY. Sets may be obtained by addressing the editor at 42 Oakwood, Asheville, North Carolina. Price of a complete set is now $4.50, transportation prepaid.

Agricultural Chemistry.—Plant physiologists are deeply indebted to Dr. C. A. BROWNE for his splendid work on sugars published many years ago. Now he increases our debt to him by providing an excellent *Source Book of*

Agricultural Chemistry. It is a very illuminating and richly historical volume from which one may learn much about the background of agricultural practices and theories. The story begins in antiquity, the first chapter bearing the title: Agricultural chemistry in ancient times. The second chapter gives an account of the developments and writers during the period of alchemy and iatrochemistry. Then comes the more accessible work, published during the early period of the Royal Society of London; ROBERT BOYLE, FRANCESCO REDI, JOHN MAYOW, ROBERT HOOKE, NEHEMIAH GREW, JOHN WINTHROP the younger, JOHN CLAYTON, STEPHEN HALES, and JETHRO TULL are great figures of this period. Chapter four carries the presentation into the early phlogiston period, and chapter five to the later part of the phlogiston era, where the dominant names are those familiar to students of plant physiology, BLACK, PRIESTLEY, INGEN-HOUSZ, SENEBIER, CAVENDISH, and SCHEELE. Then comes the chemical revolution through the brilliant interpretations of LAVOISIER, the accurate analyses of DE SAUSSURE, the work of THAER and EINHOF, CHAPTAL, HERMBSTÄD, HUMPHREY DAVY, and DE CANDOLLE.

The history closes with the great leaders who, about the middle of the 19th century, became moderns in the true sense. WIEGMANN, SCHUBLER, SPRENGEL, BOUSSINGAULT, MULDER, and LIEBIG are the great contributors, with whose work the author ends the source material.

As one who has found the original books of high inspirational value, a proud possessor of the land-mark works of many of these authors, the writer recommends this source book most highly. The publishers are The Chronica Botanica Co., Waltham, Massachusetts; and G. E. Stechert and Co., New York. The price quoted is $5.00 per copy. Send orders to the publishers.

Traces of Metals.—Plant physiologists will welcome the publication of a distinctive analytical work entitled: *Colorimetric Determination of Traces of Metals,* by E. B. SANDELL, the University of Minnesota. It is volume III in a series of monographs on analytical chemistry and its applications. The first few chapters are general, and deal with principles, methods, and reagents. These four chapter headings are as follows: Trace analysis; methods for the separation and isolation of traces of substances; colorimetry and spectrophotometry in trace analysis; and general colorimetric reagents.

The special part, which forms the major portion of the book, gives the methods for specific elements. There are 45 chapters in the special part of the book, each usually dealing with a single element, or very closely related elements. The following are the chapter headings: Aluminum; antimony; arsenic; barium; beryllium; bismuth; cadmium; calcium; cerium; chromium; cobalt; columbium and tantalum; copper; gallium; germanium; indium; iridium; iron; lead; lithium; magnesium; mercury; molybdenum; nickel; osmium; palladium; platinum; potassium; the rare earth elements; rhenium; rhodium; ruthenium; scandium; silver; sodium; thallium; tin; titanium; tungsten; vanadium; zinc; and zirconium. It is a comprehensive treatment, and should be very helpful in connection with trace element nutrition studies.

With author and subject indexes, it comprises 487 pages. The price quoted is $7.00 per copy. Orders should be sent to Interscience Publishers, Inc., 215 Fourth Ave., New York 3, N. Y.

Annual Review of Physiology.—Physiologists will welcome the appearance of volume VI of the *Annual Review of Physiology*, which in spite of war conditions continues its vital service. There are 19 reviews, and the titles are a sufficient characterization of the contents of the volume. The titles are as follows: Developmental physiology, by V. HAMBURGER; growth, neoplastic growth, J. FURTH; physiological effects of heat and cold, J. E. FIELD, 2nd, and V. E. HALL; tissue water and electrolyte, D. C. DARROW; energy metabolism, M. KLEIBER; respiration, T. BERNTHAL; the physiology of the skin, S. ROTHMAN and P. FLESCH; digestive system, B. SLUTZKY and A. C. ANDERSEN; liver and bile, L. A. CRANDALL, Jr.; kidney, E. BRAUN-MENENDEZ; blood, A. J. QUICK; heart, R. ASHMAN; conductive and synaptic transmission in the nervous system, D. P. C. LLOYD; visceral functions of the nervous system, B. A. MCSWINEY; vision, F. W. WEYMOUTH; metabolic functions of the endocrine system, F. G. YOUNG; reproduction and its endocrine system, A. S. PARKES; physiological psychology, W. FREEMAN and J. W. WATTS; and industrial physiology, E. SIMONSON.

These reviews sketch the trends of physiological research and interpretation. They are an invaluable adjunct to the research laboratory and library. Everyone who engages in investigation must perforce apply himself to some very restricted phase of his field; but it is also necessary to keep a broad and well-balanced outlook upon the whole field, as of physiology. The *Annual Review of Physiology* enables one to keep abreast of the total advances in this region of research.

The reviewer is impressed afresh by the wide difference in the interests of animal and plant physiologists, in spite of the fact that we are all dealing with living organisms, biological material. It ought to be obvious that there are difficulties, probably insuperable difficulties, in the way of a "general biology." While there are a few things in common in the two fields, zoology and botany, there is not enough to permit of organization into a single science, without which organization one has no right to speak of science.

The development of animal physiology must go on along the lines indicated in this volume, with its excellent summaries; and animal biology must always be concerned with digestive systems, livers, kidneys, blood, heart, brains, nerves, special organs of sense, endocrine glands, etc. But these belong, not to general biology, but to specialized animal biology.

The editors and authors engaged in this enterprise of reviewing the advances in these specialized fields are performing a necessary service with great skill and understanding. They deserve the thanks and congratulations of all of us as beneficiaries of their devotion.

Orders for Volume VI of this series should be sent to Annual Reviews, Inc., Stanford University, California. The price is maintained at $5.00 per copy.